

Correlation between Structure and Function in the Neuromuscular System of the Earthworm, *Eisenia foetida*, Sav.

PART I. Correlation between Development of Behavior and
Neuromuscular Differentiation in Embryos of *Eisenia*
foetida, Sav.

PART II. Effect of the Central Nervous System on Responses
to Light in *Eisenia foetida*, Sav.

By
CLIFFORD LADD PROSSER

A DISSERTATION
SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE
JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN 1932

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CORRELATION BETWEEN DEVELOPMENT OF BEHAVIOR AND NEUROMUSCULAR DIFFERENTIATION IN EMBRYOS OF EISENIA FOETIDA, SAV.

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FIVE PLATES (TWENTY-FIVE FIGURES)

INTRODUCTION

The subject of embryonic behavior as correlated with morphological development has yielded important facts concerning functional interrelations among various parts of the nervous and muscular systems in the organisms in which it has been investigated. These results have recently been summarized by Coghill ('29) and Langworthy ('32). These accounts state that, in general, the first movements of many vertebrate embryos are of gross regions of the body, that reflexes are individuated from general behavior complexes partly by the addition of inhibitory systems, and that the development of behavior proceeds in a cephalo-caudad direction. The appearance of different types of movement depends upon the differentiation of definite structures.

Some of the outstanding movements of many invertebrate embryos have been studied (Preyer, 1883) but apparently the only invertebrate in which an attempt has been made to trace the development of behavior is *Limulus* (Pearl, '04). The earthworm is admirably adapted for such a study, because its embryos are readily available, it undergoes direct development, and its morphological development has been carefully

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studied. A review of its embryology is essential to an understanding of the correlation between structure and function in embryos of this organism.

It was early recognized that earthworms were hermaphroditic and Cuvier (1817), Meckel (1815), and Montegre (1815) said that they were viviparous. Their eggs were observed by Leo (1820), Dufour (1825, 1828), and Dugès (1828), but their function was not clearly understood.

Detailed accounts of the embryology of earthworms were given by d'Udekem (1856), Ratzel (1869), Ratzel and Warschawsky (1869), Kowalewsky (1871), Hatscheck (1878), Kleinenberg (1879, 1882), Wilson (1887, 1889), Bergh (1886, 1890), Vejdovsky (1888-1892), Bourne (1894), and Staff ('10). Wilson studied several species and presented a very understandable account of their development. His results substantiated most of those of the other workers and may be summarized as follows: The eggs are laid in small oval capsules which are filled with albumen in which they float. Cleavage is unequal and gastrulation is by embolic invagination. In early gastrulae two mesodermal bands arise from posterior primary mesoblasts. These mesodermal bands proliferate laterally and extend into the unpaired prostomium. The mesoderm is of two sorts, trunk mesoblast in which coelomic cavities are formed, and migratory mesoblast or mesenchyme which constitutes a loose muscular network beneath the entire ectoderm. The trunk mesoblast forms an outer pair of double germ bands which give rise to muscles, septa, blood vessels, peritoneal epithelium, reproductive organs, and the inner part of the nephridia. Ventral to the mesoderm lie the middle and inner germ bands, each derived from a large cell or teloblast which originates at the anterior end and is gradually pushed backward. Both of these bands are ectodermal. Each of the inner or ventral rows forms half of the ventral nerve cord and is called a neural band. The middle bands, like the mesodermal bands, appear double and are called nephric rows; they contribute to the outer part of the nephridia and to the setigerous glands. Between the neural and nephric bands arise narrow mesodermal rows of longitudinal muscles, called accessory or embryonic muscles; the circular muscles, derived from the lateral bands, extend at the sides of the embryo. The suprapharyngeal ganglion or brain arises by the fusion of two masses of neuroblasts at the anterior end of the neural rows which are continuous from

brain to cord. The inner or neural germ bands then fuse ventrally, from the anterior end backward. Later, the first three ventral ganglia unite as the subpharyngeal ganglionic mass. The ventral nerve cord receives a mesodermal sheath, the fibrillar substances are differentiated gradually and the giant fibers appear relatively late. The funnel and investing cells of the nephridia are mesodermal, the tubular body is derived from the ectodermal nephric bands. Proctodaeal invagination occurs late in development and the embryo is nearly ready to hatch before the digestive tube is complete. The ventral setae originate in setigerous glands or sacs which alternate with the nephridia in the nephric rows. Lateral setigerous glands are also formed from the nephric bands, lateral to the ventral ones.

The only statements concerning movements in embryos of earthworms are that emergent worms are able to crawl and burrow (Dufour, 1828) and that at very early stages they swallow large amounts of albumen (Hatschek, 1878 et al.).

The results obtained in an attempt to apply to the earthworm, *Eisenia foetida*, the methods used with embryos of higher forms are presented in the following pages.

SPONTANEOUS MOVEMENTS OF EMBRYOS OF EISENIA WITHIN THE EGG CAPSULES

Egg capsules containing embryos of *Eisenia foetida* were collected in a pile of decaying leaves or in a compost pile. One egg capsule containing several embryos in very early stages of development was put into each of four watch glasses. The capsules were then covered with pieces of moist leaves and kept in the laboratory at 20 to 24°C. They were removed from their respective watch-glasses and observed under a binocular dissecting microscope at 1- or 2-day intervals until the embryos hatched.

A typical record of the movements and appearance of the embryos in one egg capsule follows:

First day. Two embryos were present in cleavage or blastula stages. No movements were observed.

Second day. The embryos were in gastrula stage (fig. 1). These gastrulae floated in a mass of albumen with which the capsule was filled and rotated slightly as a result of the activ-

ity of cilia on the ventral surface and in the stomodaeum. No movements of the body were observed.

Third day. The albumen in the capsule was being swallowed and mesodermal bands were differentiated ventrally (fig. 2). The region above the stomodaeum contracted slightly. Ciliary movement continued.

Seventh day. Several segments were present in front of the albumen in the archenteron, and indentations, which indicated the beginning of segmentation, appeared below this albumen (fig. 3). Contractions in the stomodaeum resulted in the swallowing of much albumen. Strong contractions also occurred locally at various points over the surface of the body, and these initiated peristaltic waves which passed either forward or backward.

Tenth day. There were approximately seven segments anterior to the albumen in the archenteron or gut (fig. 4). The embryos had consumed all the albumen in the egg capsule. The contractions of the stomodaeum and of various parts of the body surface persisted, and contractions in the neck region caused the head to turn from one side to the other.

Thirteenth day. Additional segments were differentiated in the anterior end. The head flexion was rhythmic and lateral extension was accompanied by considerable contraction in the neck; this contraction, as well as local contractions over the surface, initiated peristaltic waves.

Seventeenth day. Approximately ten to twelve segments were present anterior to the albumen in the gut, mesoderm covered all but a small postero-dorsal region of this albumen (fig. 5). The head extended forward more frequently than sideward. The body rolled and twisted considerably.

Twentieth day. The entire body was segmented (fig. 6). The head circled and extended forward. Both ends turned and twisted so that the animals formed a tight coil.

Twenty-first day. Both embryos hatched. They appeared like normal adult worms, but were cream-colored. They crawled and burrowed into humus.

Essentially the same results were obtained in the observations on the three other capsules. These results indicate that approximately $2\frac{1}{2}$ to 3 weeks are required from the time of gastrulation to hatching. They also indicate that embryos of *Eisenia foetida* move spontaneously in different ways according to the stage of development, that early types of move-

ment are replaced by later ones, and that the order of appearance of these types of spontaneous movement is as follows: ciliary activity, contraction of the stomodaeal region, local contractions over the surface of the body, flexions of the head, extension of the head, and coiling of the entire body. These results also indicate that the amount and kind of movement depends, in part at least, upon the number of differentiated segments in which no undigested albumen is present.

STRUCTURE AND BEHAVIOR IN EMBRYOS REMOVED FROM THEIR EGG CAPSULES

Egg capsules containing embryos in various stages of development were taken to the laboratory and within a few hours were opened by means of fine scissors and needles; the embryos were then allowed to drop from the capsule into a watch glass containing either physiological salt solution or tap water. The solution used in most of the experiments consisted of NaCl 0.7 gm., KCl 0.025 gm., CaCl_2 0.03 gm., and H_2O 100 cc., with a hydrogen ion concentration of approximately pH 6.2. The embryos lived in this solution for a number of hours and apparently moved and responded in a normal manner.

Most observations were made within 1 or 2 hours after removal of the embryos from the egg capsule. All experiments were performed in a dark room where the embryos were kept until ready for use. The temperature averaged 22 to 23°C. for the first 100 specimens and 23 to 25°C. for the remainder. All observations were made with the aid of a binocular dissecting microscope.

The observations were made as follows: A piece of tissue paper approximately 2.5 cm. square was put into a Syracuse watch glass containing salt solution approximately 2 mm. deep; then an embryo was placed on the paper which served to facilitate movement.

Each embryo was first observed in weak illumination as to kind and extent of spontaneous movements, then light from a 500-watt projection lamp, 90 cm. from the mirror of the micro-

scope was, by means of the mirror, focused upon the embryo. If responses occurred upon sudden illumination, the reaction time was noted. Now, by means of an opaque screen one-half of the field was darkened and the embryo placed in the illuminated part of the field parallel to and close to the shadow. If it turned toward the shadow, the procedure was repeated with the other half of the field darkened. After this, the embryo was usually illuminated from above and then touched with a fine needle in the dorsal, the lateral, and the ventral parts of the anterior, the median, and the posterior regions, the magnitude of the stimulus being kept as nearly constant as possible, although it was by no means quantitatively measurable. However, since the purpose was to test coordination between various regions, the fact that the stimulus was not measured was of little significance as long as it was kept fairly constant. After the spontaneous movements and the responses were recorded, the embryo was returned to its respective watch glass, then a camera lucida outline ($\times 25$) was made, after which the embryo was dropped into a shell vial containing fixing fluid.

Eight different fixatives were tried, but best results were obtained with Flemming's strong solution. A few specimens were stained with borax carmine and mounted in toto, but most of them were sectioned and stained with Heidenhain's haematoxylin and eosin, which gave better results than any of the other stains tried. No success was obtained with methylene blue or silver nitrate as nerve stains, but good results were obtained with the gold chloride technique of Gairns ('30).

The behavior and morphological data thus obtained for each specimen were summarized on a card. Each card contained the following items: specimen number, date and temperature (usually), spontaneous movements, responses to tactile stimuli, responses to light, and a description of structure with specific reference to nerves and muscles.

Records for approximately 300 individuals were made. These were fairly equally distributed among all stages of

development from gastrulae to worms which were ready to hatch.

After a study of the cards containing these records, they were divided into six groups representing different stages of development of the embryos with respect to morphology and behavior. Some of these groups were further subdivided. This distribution of the embryos is entirely arbitrary. In reality, they pass through a gradual series of different modes of behavior corresponding with their morphological development. In all stages overlapping from one group to another is found.

The following pages contain an account of the results obtained in a detailed study of these records. Records of typical individuals in each group will be presented, the variation within each group will be indicated, and the correlations between structure and function will be considered. The descriptions of behavior and structure in the typical records do not usually refer to the same, but to two similar specimens.

Stage of non-muscular movement (fig. 1)

Behavior. No organized movements of the body of this gastrula and no responses to any stimuli were observed. Ventral cilia, however, beat backward with such force that the embryo rotated slowly when it rested on its side; and cilia in the stomodaeum created a slight vortex in which particles were drawn in and swept out of the mouth.

Structure. The stomodaeum of this gastrula was deeply invaginated and around it were a few scattered spindle-shaped cells which were interpreted as being muscle cells of the migratory mesoderm. Mesodermal bands extended the entire length and were fairly compact; inner germ bands (nephric and neural) ended in teloblasts somewhat anterior to the posterior tip, and all of them were distinctly separate except the neural bands which were beginning to fuse above the stomodaeum to form the brain. Neuroblasts were continuous from brain to ventral bands. Some of the dorsal epithelial cells were spindle-shaped, but most of the epithelial

cells were columnar; large ectodermal cells in a double ventral row were ciliated as were the cells lining the stomodaeum. A slight amount of albumen was present in the archenteron.

Essentially the same results were obtained in six individuals, except that in two which were more mature, slight contractions appeared about the stomodaeum, usually above it; these contractions appeared as slight indentations in the contracting region. A cross section of one of these specimens, cut just behind the stomodaeum, is represented in figure 7, in which the separate germ bands are clearly shown. A schematic diagram illustrating the condition of the neural germ bands is given in figure 20.

Stage of stomodaeal contractions (fig. 2)

Behavior. (Three embryos in one egg capsule.) The region around the stomodaeum contracted, as shown by slight indentations in this region. In one embryo the contractions were dorsal to the stomodaeum and were irregular; in the other two, the contractions involved the entire stomodaeal region and in these the contractions were rhythmic, averaging twenty per minute. They were strongest on the dorsal side where they appeared to originate. These stomodaeal contractions varied in strength. The strongest ones often exerted such a pressure that the whole embryo rounded up like a ball or was drawn out in the shape of a pear. The stomodaeal and ventral cilia were active. No responses were given to tactile or photic stimulation.

Structure. Lobate or columnar epithelial cells on the ventral surface intergraded with spindle-shaped or squamous ones on the dorsal surface, particularly in the anterior end. Spindle-shaped muscle cells formed a ring around the stomodaeum and were found beneath the epithelium in the anterior end and ventrally in the middle of the body. The neural bands were united above and were adjacent below the stomodaeum, thus forming a ring around the latter. These neural bands consisted of many crowded neuroblasts, no processes being present. Mesodermal bands were fairly compact at the sides and the ventral lip was enlarged as a gland-like lobe.

These records are typical of twenty-four specimens studied, except that in the less mature, the stomodaeal contractions were weak, irregular, and dorsal to the stomodaeum, and in the more mature they were strong, rhythmic, and involved more of the region around the stomodaeum. In the more mature specimens, also, local contractions occasionally appeared in different parts of the surface of the embryo, particularly in the antero-dorsal region. The contractions of the stomodaeum in twenty-four specimens averaged sixteen per minute. A slightly oblique section of the anterior end of one of the younger specimens is represented in figure 8 which shows the continuity of brain and ventral cord before the neural bands fuse ventrally. A cross section of a more mature specimen just posterior to the subpharyngeal ganglion in which the bands are fused is represented in figure 9 and a sagittal section of the anterior end of one in figure 15. The typical structure of a member of this group is represented in figure 21 in which the neural bands are shown to be double throughout, except above and below the stomodaeum.

The development of rhythmic stomodaeal contractions involving the entire region around the stomodaeum from occasional contractions dorsal to the mouth is apparently correlated with the increase in mesenchymatous muscle cells from the migratory mesoderm around the stomodaeum and with the formation of spindle-shaped or squamous epithelial cells over the dorsal surface.

Stage of local surface contractions (fig. 3)

The embryos in this stage were divided into two groups. The following is typical of a member of the younger group:

Behavior. The stomodaeal contractions were regular twenty to twenty-four per minute; they involved all of the region surrounding the stomodaeum. Many local contractions occurred, especially on the dorsal surface and in the first three or four segments. These contractions initiated peristaltic waves which passed down one side or around the entire body. Irregularity of stomodaeal and local contractions

caused some turning, particularly at the anterior end. When stimulated with a needle below the stomodaeum, this embryo responded by local contraction at the point stimulated. No responses were given elsewhere.

Structure. The brain was separated from the prostomial tip by a few loose mesenchymatous and muscle cells. The neural bands were fused approximately one-half the length of the embryo. Neuroblasts appeared to be migrating from the commissural region, leaving a few fibers there. A very few fibers were also present in the brain and subpharyngeal ganglion. The mesodermal bands contained rudiments of the coelom and the inner mesoderm was condensing at the sides of the anterior end of the ventral cord as the first cells of the embryonic longitudinal muscles.

Essentially the same results were obtained in thirty-five specimens. In the more mature of these, peristaltic waves, initiated by both stomodaeal and surface contractions, passed either anteriorly or posteriorly, and in some, contractions in response to tactile stimulation occurred at points at the sides of as well as below the stomodaeum. The structure of these specimens is indicated in figures 10 and 16. These figures show a few muscle cells which constitute an accessory longitudinal strand of muscles close to the fused ventral cord and a few spindle-shaped cells in the lateral mesoderm which will contribute to the circular muscles.

The more mature embryos in this stage differed in behavior and structure from the earlier ones somewhat as follows:

Behavior. Stomodaeal contractions were less regular than in younger embryos of this stage. Local contractions on the surface initiated peristaltic waves which passed in either direction. Strong contractions in the neck region caused head turning. In response to tactile stimuli, local contractions occurred anywhere anterior to the albumen in the gut and on the ventral surface about one-half the length of the embryo. The responses in the anterior region were stronger ventrally than laterally and dorsally.

Structure. The neural bands were fused in the anterior two-thirds of the embryo; nerve fibers were present in the brain, in the pharyngeal commissures, and in one-third the length of the cord, the longitudinal fibers extending a little behind the transverse ones. Mesenchymatous cells separated the brain from the tip of the prostomium. Circular muscle cells were present beneath the entire epithelium anterior to the albumen in the archenteron and beneath the ventral epithelium one-half the length of the embryo. Two narrow bands of longitudinal muscles closely bounded the nerve cord and extended back slightly farther than the circular muscles. No setae were present, but ventral and stomodaeal cilia were distinct.

Approximately the same results were obtained in twenty-six specimens. They varied in that some responded to tactile stimulation by local contraction only in the region anterior to the albumen in the archenteron, while others responded to tactile stimulation in this region and also on the entire ventral surface. Frequently these contractions were so strong that the anterior end was turned toward the side stimulated. Structurally the specimens varied in that the number of segments differentiated anterior to the albumen ranged from two to five and in that in some, the circular muscles extended nearly around the embryo but were confined to the anterior region, while in others, in addition, they extended on the ventral side nearly to the posterior end. In all, the longitudinal muscles extended a little farther back than the circular muscles, but the circular muscles extended toward the dorsal side farther than the longitudinal muscles. They varied also in that in some, nerve fibers were present only in the anterior quarter of the cord, while in others they were present in three-fourths of the cord, and in still others (a few of the most mature) lateral nerves extended from the subpharyngeal ganglion.

The two groups of specimens just considered constitute the stage in development of the beginning of spontaneous contractions on the surface of the embryo, particularly in the

region of the neck (segments two to six) and of the appearance of the first response to tactile stimulation, namely, local contraction at the point stimulated. Figure 22 is a schematic diagram of an embryo at this stage. It shows that the longitudinal and the circular muscles at the anterior end extend entirely around the embryo, that on the ventral surface they extend approximately two-thirds the length of the embryo, and that nerve fibers in the cord extend nearly the same distance posteriorly as do the circular muscles.

The spontaneous local contractions apparently depend upon the differentiation of spindle-shaped epithelial and loose muscle cells. Contraction in response to stimuli, however, is correlated with the appearance of distinct muscle cells of the circular and longitudinal layers. Apparently the response is to direct stimulation of the contracting cells. There is no evidence that the nerve fibers in the anterior part of the cord function at this time.

Stage of flexions of the head (fig. 4)

The embryos in this stage were divided into three groups, according to maturity. The behavior and structure of the earliest is indicated in the following records:

Behavior. The head was regularly flexed to the side by the contraction of muscles in the neck region. Peristaltic waves originated either in these contractions of the neck or in local contractions over the surface. No prostomial contractions were observed. In response to local tactile stimulation of the anterior end, this end turned slightly from the side stimulated irrespective of whether the stimulus was on the dorsal, ventral, or lateral surface. Responses to stimulation of the ventral surface of the middle and posterior regions consisted of local contractions.

Structure. The ventral cord was fused throughout its entire length. Nerve fibers were present in the central and ventral parts of the brain and in approximately the anterior two-thirds of the cord. Several pairs of lateral nerves extended from the anterior ventral ganglia. The brain was

40 μ from the anterior end of the embryo and the intervening space was filled with mesenchyme and neuroblasts. Longitudinal fibers extended slightly farther back in the cord than transverse ones. Circular and longitudinal muscles were complete at the anterior end and decreased in extent backward, ending in two ventral bands at the posterior end. The nuclei of the longitudinal muscle cells were proximal and the first muscle fibers in them appeared near their outer surface. Circular muscles extended farther latero-dorsally than longitudinal muscles. A few anterior setae were present and the ventral cilia were diminishing.

Similar results were obtained in thirty-three specimens. These specimens varied in the magnitude and the frequency of the flexion of the head in response to tactile stimulation, the less mature specimens responding only to lateral, the more mature also to ventral and dorsal stimulation. In all, the head was turned in the direction opposite the surface stimulated irrespective of whether that was dorsal, ventral or lateral.² They varied structurally in that the brain was either in the first or in the second segment and in that the lateral nerves, as traced by the gold chloride method, extended for varying distances into the muscles anterior to the albumen. Figures 12 and 17 illustrate the extension of these nerves and the development of the two muscle layers.

In a specimen somewhat more mature than the one just described, the following was observed:

Behavior. There was irregular and strong swinging or flexion of the head with extension to one side; this extension involved contraction in the anterior four to five segments and this contraction initiated peristaltic waves. The strongest pull in this flexion appeared to be on the ventral side. The embryo did not rest on its ventral surface, but on its side. In response to tactile stimulation the anterior end turned away from stimulation in any part of the anterior end and from stimulation on the ventral surface one-fourth the length

² That these responses were real and were not movements which would occur without stimulation was evident from the facts that this movement of the head was more rapid and forceful than spontaneous flexion of the head, and that on stimulation a resting specimen responded by flexion away from the side stimulated.

of the albumen in the gut. Local contractions persisted in response to stimulation on the ventral surface of the middle and posterior regions.

Structure. Nerve fibers were present in approximately the anterior three-fourths of the cord and lateral nerves extended from ganglia in the anterior half. The embryonic bands of longitudinal muscles extended the entire length of the embryo; the lateral bands of longitudinal muscles extended two-thirds the length and the circular muscles three-fourths the length, the circular muscles extending farther at the sides than the longitudinal ones.

Similar results were obtained in thirty-six specimens. In these specimens lateral nerves extended back slightly farther than the limit of the region which could be stimulated with the production of the flexion of the head as a response.

In the most mature of the specimens in this stage the following was observed:

Behavior. The anterior end rotated rhythmically from side to side and frequently extended some distance laterally; this extension caused a contraction in the neck region which initiated peristaltic waves. These waves sometimes also originated in local surface contractions. Flexions of the anterior end changed the tension in other parts of the body and thus induced the posterior end to turn in the same direction as the head. The anterior end turned away from stimulated points on the ventral and lateral surface in the anterior two-thirds, and from stimulated points on any surface anterior to the albumen, and local contractions persisted in response to ventral and lateral stimulation in the posterior and middle regions.

Structure. The brain was between the second and third segments. Longitudinal fibers were present the entire length of the cord and transverse fibers were present in all the ganglia except the last one. Lateral nerves were present in the anterior three-fourths of the embryo and a few sensory cells were present in the epithelium of the anterior end. Both circular and longitudinal muscle layers extended completely

around the anterior end and back along the ventral surface except for an epithelial bud at the posterior end; the circular muscles extended a little farther at the sides than did the longitudinal muscles.

Similar results were obtained in twenty-five specimens. In these specimens the head flexion in response to stimulation varied according to what the anterior end was doing at the time of stimulation. If it was turning toward the side away from the region stimulated, it continued in this direction, but the rate and extent of movement increased. If it was directed straight ahead, it turned away from the side stimulated. If it was turning toward the side stimulated, it either stopped turning, or it completed the phase and then swung back, or it immediately reversed the direction of turning and at the same time turned either dorsally or ventrally; the last was the most frequent response.

The three groups of embryos just considered constituted the stage when the third type of spontaneous movements and the second type of response, namely, flexion of the anterior end, developed from a weak irregular turn to a strong extension. As a response, it was given to stimulation in a progressively increasing area. The structure of specimens in this stage is indicated by figure 23. They were distinguished by a rapid increase in the number of segments free from albumen, by the lateral extension of muscles, by the extension of lateral nerves, and in the more mature specimens, by a few sensory cells in the anterior epithelium. In some, the lateral nerves were traced from the ganglia slightly posterior to the sensitive area. The flexions of the head, therefore, appear to be correlated with the increase in free segments anterior to the albumen, with the development of longitudinal and circular muscles and with the appearance of lateral nerves.

Stage of weak crawling (fig. 5)

Behavior. Crawling movements were executed by the anterior end, that is, by the segments which contained no albumen. The action in these segments resulted in dragging along those

which contained albumen. Lateral flexion of the head was rhythmic and was often accompanied by flexion of the tail in the same direction. The head was extended forward and placed firmly upon the substratum. In response to tactile stimulation the anterior end turned from the side stimulated irrespective of the point stimulated, except that no response occurred to stimulation on the dorsal surface of the segments containing albumen and on the surface of the epithelial bud at the posterior end. The posterior third, or tail, turned in the same direction as the anterior end, or head, if the stimulus was strong and applied near the posterior end, but it did not usually begin to turn as soon as the head, sometimes following it by as much as a second. Stimulation in the anterior end evoked a stronger and wider flexion of the head than stimulation in the middle, and stimulation in this region evoked greater response than stimulation in the posterior region. Similarly, only the first few segments responded to a weak stimulus applied in the middle of the body, the neck region of six to ten segments responded to a moderate one, and the entire anterior end back to the point of stimulation turned when a strong stimulus was applied. In response to sudden illumination, the anterior end contracted strongly and the average reaction time was 2.7 seconds. When parts of the embryo were progressively illuminated from the posterior end forward, no response was given until the prostomium was reached. The embryo also rolled and crawled from an illuminated field into a shaded region.

Structure. Approximately ten segments were present anterior to the albumen. The brain was in the third segment and cephalic nerves bearing enlargements extended from the brain to the prostomium. Nerve fibers extended the entire length of the cord and lateral nerves extended from all the ganglia. Both longitudinal and circular muscles completely surrounded the body except for a small postero-dorsal region over the albumen and the epithelial tail bud. Setae were present in the anterior half, and the ventral cilia had nearly disappeared.

Similar results were obtained in fifty-one specimens. They varied, however, in that the flexion of the head in the more mature specimens was less rhythmic and more irregular and exploratory than in the less mature; that is, the head paused and waved about at the limits of the flexion in either direction, and in that the less mature specimens did not crawl. They did not crawl because the forward extension, placing of the anterior end upon the substratum, and exploratory flexion did not involve enough segments, as indicated by the fact that when they were transected in the middle, the anterior half crawled and showed burrowing tendencies, while the posterior end gave no movements except a few local contractions. Hence, the capacity for crawling exists in the anterior end long before the worm as a whole can crawl. These specimens also varied in the magnitude of the flexion of the anterior end in response to tactile stimulation and in the extent to which the posterior end was dependent upon the anterior end in response to posterior stimulation. This dependence was demonstrated in specimens which were cut into two equal halves; the posterior half responded by tail flexion, but when this was again transected, the remaining posterior part gave no responses except local contractions, indicating that transverse connections were not made at this point. These specimens varied finally in that the less mature did not respond to increased illumination, while in the more mature specimens the anterior end contracted strongly when the prostomium was brightly illuminated, the reaction time in eighteen specimens, four tests each, averaging 2.8 seconds.

The structure of specimens in this stage is indicated in figures 13, 18, and 24. These figures show that the brain was in the third segment, that muscles and nerves were complete except for an area in the postero-dorsal region over the albumen and that prostomial nerves with distinct peripheral enlargements were present. Hesse (1896) and Hess ('25) demonstrated photoreceptors in these enlargements in adult worms, and it is very probable, although not definitely demonstrated, that photoreceptors were present in them at this

stage. Crawling and the response of the posterior end to stimulation are apparently correlated with the development of muscles and nerves and with the differentiation of segments unhindered by the presence of albumen in the gut. Response to light is probably correlated with the differentiation of photoreceptors and certainly with the presence of prostomial nerves and fibers in the brain.

Stage of normal crawling (fig. 6)

Behavior. In crawling the head was extended forward, was frequently swung about in an exploratory manner, was, from time to time, firmly placed on the substratum and attached by setae. By its extension peristaltic waves were initiated and by the alternate contraction and relaxation of circular and longitudinal muscles these waves passed backward. When placed upon loose humus the embryo burrowed into it immediately. In response to tactile stimulation anywhere end-to-end contractions occurred.³ The posterior end sometimes responded by flexion independently of the anterior end. In response to photic stimulation the anterior end strongly contracted, the average reaction time in five exposures being 2.5 seconds. The embryo oriented negatively in direct illumination, and other parts of the body in addition to the prostomium were sensitive.

Structure. Nerve fibers in the ventral cord, lateral nerves, both longitudinal and circular muscles, and setae were complete. Numerous receptors were present in the epithelium, particularly in the anterior end. The diameter of the cord was fairly uniform and the giant nerve fiber in the dorsal part of the cord extended its entire length. Only the cilia on the dorsal surface of the pharyngeal pouch persisted.

Similar results were obtained in forty-seven specimens. They varied in the degree of coordination in crawling; in the less mature both ends and both sides of the body were

³ This is the last type of response to appear. It consists of a violent swinging of both ends of the body in the same direction, with the result that the entire worm is twisted into a loose coil.

involved. In these specimens transverse connections were complete at the posterior end, as indicated by the observation that when embryos were transected in the middle, the ends of both halves turned from the side stimulated, and when the posterior piece was again equally transected, the remaining posterior part responded by a flexion of the tip. They also varied in their reaction time to sudden illumination, the average for thirty-one specimens, four or five tests each, being 2.6 seconds.

The structure of specimens in this stage is indicated in figures 14, 19, and 25. Lateral nerves, circular and longitudinal muscles were complete over the entire surface, but the layer of longitudinal muscles was narrower in the postero-dorsal region than elsewhere. In the less mature specimens the giant fiber was present only in the anterior end of the cord and in the more mature specimens it was present throughout the cord.

These facts indicate that the execution of normal crawling and burrowing movements depends upon the development of circular and longitudinal muscles and lateral nerves in all parts of the body and the diminution of the amount of albumen in the gut and that end-to-end contraction is correlated with the differentiation of the giant fiber in the ventral nerve cord.

Summary

The following table summarizes the results presented in the preceding sections concerning the progressive changes with respect to spontaneous movements, responses to tactile and photic stimulation, and structural details:

<i>Spontaneous movements</i>	<i>Responses</i>	<i>Structure</i>
Stage of non-muscular activity		
Stomodaeal cilia create vortex, ventral cilia beat backward, may cause rotation. A few spontaneous contractions around stomodaeum.	No responses.	Cilia in ventral band and lining stomodaeum. A few spindle-shaped muscle cells beneath anterior epithelium; some epithelial cells spindle-shaped. Germ bands double except above stomodaeum.
Stage of stomodaeal contractions		
Contractions around stomodaeum, irregular in frequency, magnitude, and position; later they become rhythmic, approximately 18 per minute. Irregular local spontaneous contractions over surface of body.	No responses.	Much albumen present in archenteron. Neural bands fused at brain, fusing at subpharyngeal ganglion. Many spindle-shaped mesodermal muscle cells. Many dorsal spindle-shaped epithelial cells.
Stage of local surface contractions		
Local contractions on surface often cause rolling of body; may initiate peristaltic waves which may pass to anterior or posterior end. Stomodaeal contractions irregular, may initiate peristalsis. Strong contractions in segments anterior to albumen (neck).	Local contractions in response to tactile stimulation, first ventral, then lateral and dorsal to stomodaeum, later back beneath albumen.	Cord fused two-thirds length; brain begins to move back from prostomium. Few nerve fibers in pharyngeal commissures, then in subpharyngeal ganglion and brain, later longitudinal fibers half length of cord, transverse just anterior to them. Longitudinal muscles at sides of cord, extend posterior to circular muscles but not so far laterally. Setae in setigerous sacs, anterior ones emerge.

<i>Spontaneous movements</i>	<i>Responses</i>	<i>Structure</i>
Stage of flexions of the head		
Peristaltic waves originate in strong neck or surface contractions.	Local contractions entire length ventrally.	Cord single throughout, fibers nearly to end; diameter decreases posteriorly.
Flexion of head by unequal neck contraction, becomes rhythmic.	Anterior end turns from stimulation anterior to al-bumen, then in increasing ventral area.	Lateral nerves to point just posterior to sensitive area.
Lateral extension of head.	If head is rotating when stimulated, it stops, reverses, turns up or down.	Sensory cells appear in anterior epithelium.
Posterior and anterior ends often turn in same direction.		Circular and longitudinal muscles entire length ventrally, complete around anterior end. Setae anterior to al-bumen. Ventral cilia disappear.
Stage of weak crawling		
Head extended forward; extension and lateral flexion start peristaltic waves.	Anterior end turns from stimulation anywhere except postero-dorsally over al-bumen.	Fibers entire length of cord, lateral nerves from all but last ganglia. Brain in third segment. Giant fiber appears in anterior end of cord.
Flexion of head more exploratory than rhythmic.	Posterior end turns with head from posterior stimulation.	Cephalic nerves with peripheral enlargements.
Head placed on substratum and attached by setae.	Anterior end contracts in response to increased illumination.	Most of ventral cilia absorbed.
Crawling successful in anterior part.		
Stage of normal crawling		
Normal crawling by extension, exploratory flexion, placing of head, peristalsis and action of setae.	Both ends turn in same direction in end-to-end contraction in response to tactile stimulation.	Longitudinal and transverse fibers, giant fiber entire length of cord.
Burrowing into humus.	Contractions approximately 2.5 seconds after increased illumination. Orientation to light.	Lateral nerves complete. Many sensory cells in epithelium. Circular and longitudinal muscles complete. Setae entire length.

DISCUSSION

What is the significance in the life of the organism of the movements which were observed in embryos of *Eisenia* and what general correlations between structure and function can be drawn?

The first spontaneous movement, ciliary activity and consequent rotation, is dependent upon the presence of ventral and stomodaeal cilia and is lost as these cilia disappear. The action of the ventral cilia cannot function in feeding because the beat is backward, but it probably serves to maintain homogeneity in the albumen in the egg capsule, to keep the embryo suspended, and to prevent accumulation of waste products and diminution of oxygen immediately around the embryo. It is likely that the stomodaeal cilia aid in the intake of albumen, although their effect must be very slight because very little albumen is present in the archenteron before stomodaeal contractions begin.

The first local contractions begin at the time when albumen is first being swallowed, before nerve fibers in the cord, lateral nerves, circular or longitudinal muscles are present. These spontaneous contractions occur first on the dorsal side of the stomodaeum, then at any point anterior to the albumen and finally over the entire surface. They give rise to both rhythmic stomodaeal contractions and peristaltic waves which begin in any part of the body and pass in either direction.

These spontaneous stomodaeal contractions appear, as suggested by Hatscheck, to cause an intake of albumen. The contractions over the body surface also aid in swallowing, but more important appears to be the resulting distribution of albumen to the endoderm cells in which large quantities of it are digested.

It is probable that the loose spindle-shaped muscle cells derived from the migratory mesoderm and located at this stage beneath the epithelium of the contracting region are responsible for these contractions. The epithelium, particularly dorsally, consists of many spindle-shaped cells which probably also contract, although they can have nothing to do

with the stomodaeal contractions because of the columnar nature of the epithelium in that region. Numerous other instances of the spontaneous contraction of muscle cells are known. Lewis ('20), for example, finds such spontaneous contractions in muscle cells and fibroblasts grown in tissue culture.

The local contractions in response to tactile stimulation begin on the ventral side of the stomodaeum, soon include all the region anterior to the albumen, and are then given in response to stimulation ventrally and laterally. When these responses begin, a few nerve fibers are present in the anterior end of the nervous system but no lateral nerves extend to the muscles. A narrow band of longitudinal muscles lies at the sides of the anterior end of the nerve cord and cells of the circular muscle layer extend farther laterally and upward. Contractions occur throughout the area covered by the circular muscles. Hence circular muscles appear to be essential for this response. Longitudinal muscles doubtless take part in the contractions in the region where they are present. The lack of coordination between different regions in this response, the lack of peripheral nervous connections, and the fact that the contractions are confined to the region stimulated, indicate that the response is myogenic.

Flexions of the head involve coordination between the two sides of the body and antero-posterior coordination in a progressively increasing region. The rhythmic spontaneous flexion of the head appears at about the same time as the flexion in response to tactile stimulation. The flexion is dependent upon the differentiation of the two muscle layers and lateral nerves. Sensory cells develop during this stage, but they frequently cannot be found in all of the sensitive region, and it is likely that free sensory endings are stimulated directly. Since longitudinal and transverse fibers are present in the cord before lateral nerves are differentiated, this response is distinctly neurogenic.

The facts that the first responses are myogenic and that these are succeeded by neurogenic responses are also in accord

with the results obtained in many other forms. Coghill ('29), for example, obtained a response from the muscles of somites to direct stimulation before nervous connections were made, and Yanase ('07) found myogenic contractions of dermal muscles in the human foetus in response to direct stimulation.

In later development both the posterior and anterior ends turn in response to stimulation of the posterior end. The facts that the posterior end responds first in conjunction with the head and that the posterior half, but not the posterior quarter, responds independently when separated from the anterior region indicate that commissural connections are not made in the posterior ganglia until relatively late. They also suggest that longitudinal coordination precedes transverse coordination, at least in this region. This view is supported by the histological evidence that longitudinal fibers in the cord are present before or posterior to the transverse ones.

Flexion of the anterior end is the forerunner of extension of the head and other crawling movements and accompanies differentiation of the muscle layers and the ganglionic connections. These early crawling movements may be of little use to the embryo while it is still within the egg capsule, but they clearly are useful after hatching. This phenomenon, the presence of the capacity for a process before this process is required, is common in the development of all organisms, for example, the neck and limb movements of mammalian foetuses.

When responses to light appear, fibers in the brain, prostomial nerves, and their peripheral enlargements which probably contain photoreceptors are present.

The latest type of response to tactile stimulation is the end-to-end contraction or coil. When this response first appears, a longitudinal giant fiber is present in the dorsal part of the ventral nerve cord. The conclusion that this fiber is necessary for these end-to-end contractions is supported by the work of Yolton ('23), who found that quick end-to-end contractions in *Eisenia* were lost when the giant fibers were cut.

It is apparent that most of the differentiation in embryos of *Eisenia foetida* proceeds from the anterior end backward. Development of cilia, fusion of neural germ bands, differentiation of nerve fibers in the ventral cord, development of the two muscle layers, extension of lateral nerves, development of giant fibers, nephridial differentiation, fusion of lateral blood vessels, and development of setae, all begin at the anterior end of the embryos and continue to the posterior end. An exception to this may be the fact that nerve fibers appear in the pharyngeal commissures before they are present in the brain. Similarly, ciliary activity, spontaneous contractions, local contractions in response to tactile stimulation, spontaneous and responsive flexions of the head, end-to-end contractions, and sensitivity to light, all develop from the anterior end backward. This antero-posterior differentiation constitutes a primary gradient, as Child ('24) has pointed out.

Differentiation also occurs laterally, from the ventral germ bands outward. The mesoderm extends laterally from the ventral bands; lateral nerves, longitudinal and circular muscles differentiate first ventrally and then laterally. Those movements which are dependent upon the differentiation of these structures also develop laterally. This ventro-lateral or ventro-dorsal differentiation constitutes the axis of a secondary gradient.

Integration in embryos of *Eisenia* is present from the beginning of development. Ciliary, muscular, and nervous activity are coordinated as the embryos develop. Increasing regions in the middle and posterior parts of the body take over the behavior pattern of the anterior end. Segmental independence is apparently a late development, and the central patterns are dominant throughout development. This is in harmony with the results obtained in higher forms. It appears that most organisms are coordinated, as is *Eisenia*, in both nervous and non-nervous processes throughout their lives.

SUMMARY

1. Embryos of *Eisenia foetida*, Sav., show different types of spontaneous movement and response to tactile and photic stimulation corresponding with their structural development.

2. In early gastrulae cilia in a ventral band beat backward and other cilia lining the stomodaeum create a vortex.

3. The first body movements are spontaneous contractions of the region around the stomodaeum, which are at first irregular and later rhythmic, approximately sixteen per minute, and spontaneous local contractions over the surface of the body, which initiate peristaltic waves. These spontaneous contractions are correlated with the presence of loose spindle-shaped muscle cells from the migratory mesoderm and spindle-shaped epithelial cells.

4. The first responses are local contractions in response to tactile stimulation at first below, then at the sides of, and above the stomodaeum, and finally along the ventral surface. They are correlated with the appearance of cells of the circular and, to some extent, longitudinal muscles.

5. Spontaneous flexion or rotation and extension of the anterior end due to unequal contractions in the neck region require the presence of circular and longitudinal muscles.

6. Flexion of the anterior end in response to tactile stimulation in an increasing area backward requires not only muscles, but fibers in the central nervous system and lateral nerves in the sensitive area.

7. Crawling and burrowing movements involving extension, exploratory swinging and placing of the head, peristaltic contraction and coordination of setae, are correlated with the extension over the entire body surface of circular and longitudinal muscles, lateral nerves, the differentiation of longitudinal and transverse fibers in the ventral cord and the development of setae.

8. Sudden contraction of the anterior end and orientation in response to photic stimulation are correlated with the development of prostomial nerves bearing enlargements which probably contain photoreceptors and with the differentiation of central nervous connections.

9. End-to-end contraction in response to tactile stimulation is correlated with the differentiation of the giant fiber in the ventral nerve cord.

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PLATES

ABBREVIATIONS USED IN FIGURES

A., albumen in archenteron	N.C., ventral nerve cord
B., brain or cephalic ganglion	Neph., nephridia
C.M., circular muscles	Neph.B., nephric band
E., ectoderm	N.F., nerve fibers in central nervous system
En., digested albumen in endoderm	O.G., oral gland
G., archenteron or gastrocoele	P., proctodaeum
L.M., longitudinal muscles	P.C., pharyngeal commissure
L.N., lateral nerves	S., stomodaeum
M., mesoderm	Se., seta
M.C., spindle-shaped muscle cell of mi- gratory mesoderm	So., somite
N.B., neural germ bands	

PLATE 1

EXPLANATION OF FIGURES

Camera lucida drawings of embryos of *Eisenia* in different stages of development. Specimens fixed in acid formalin and stained with borax carmine.

1 Stage of non-muscular activity; gastrula; shows archenteron, G; stomodaeum, S; early germ bands on ventral side. $\times 165$.

2 Stage of stomodaeal contractions; shows albumen, A, in archenteron and beginning of mesodermal extension in segmental bands. $\times 54$.

3 Stage of local contractions over surface; shows segment formation anterior and ventral to albumen. $\times 54$.

4 Stage of flexion of the head; shows increase in segments anterior to albumen, distension by albumen in archenteron, and enlargement of posterior end. $\times 37$.

5 Stage of weak crawling; shows decrease in albumen in archenteron and presence of digested albumen in gut. $\times 37$.

6 Stage of normal crawling; shows segmentation in all parts of body, decrease in albumen and beginning of proctodaeum, P. $\times 21$.

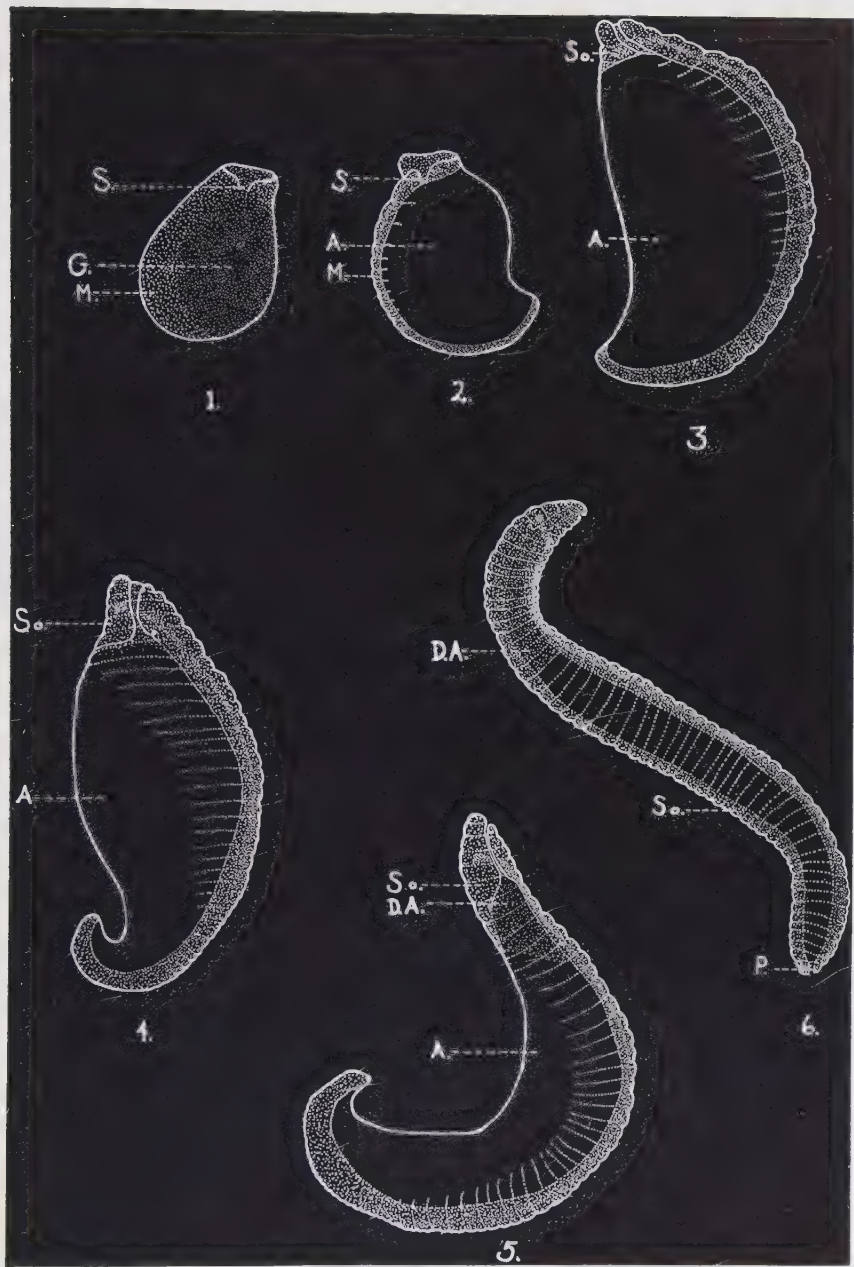


PLATE 2

EXPLANATION OF FIGURES

Camera lucida drawings of cross sections of embryos in various stages of development, slightly schematized. Sections slightly posterior to subpharyngeal ganglion unless otherwise stated. Fixation, Flemming or Gilson; stain, Heidenhain's haematoxylin and eosin.

7 Stage of non-muscular activity; showing mesodermal bands, M; early neural bands, N.B.; nephridial bands, Neph.B., and ectoderm, E. $\times 202$.

8 Stage of stomodaeal contractions; slightly oblique section through stomodaeum showing continuity of neuroblasts between brain, B, and ventral neural bands, N.B. Other sections of this specimen show thin strand connecting lobes of brain. This section also shows a few spindle-shaped muscle cells of the migratory mesoderm, M.C. $\times 492$.

9 Stage of stomodaeal contractions; showing development of germ bands before fusion of neural bands. $\times 492$.

10 Early specimen of stage of local surface contraction; showing neuroblasts in fused ventral nerve cord, beginning of longitudinal muscles, L.M., and circular muscles, C.M. $\times 492$.

11 Late specimen of stage of local contractions; cross section about one-fourth length of embryo; showing few longitudinal fibers in ventral nerve cord, N.C.; further extension of longitudinal muscles, L.M., and circular muscles, C.M.; beginning of coelom, nephridia, and setigerous sacs. $\times 165$.

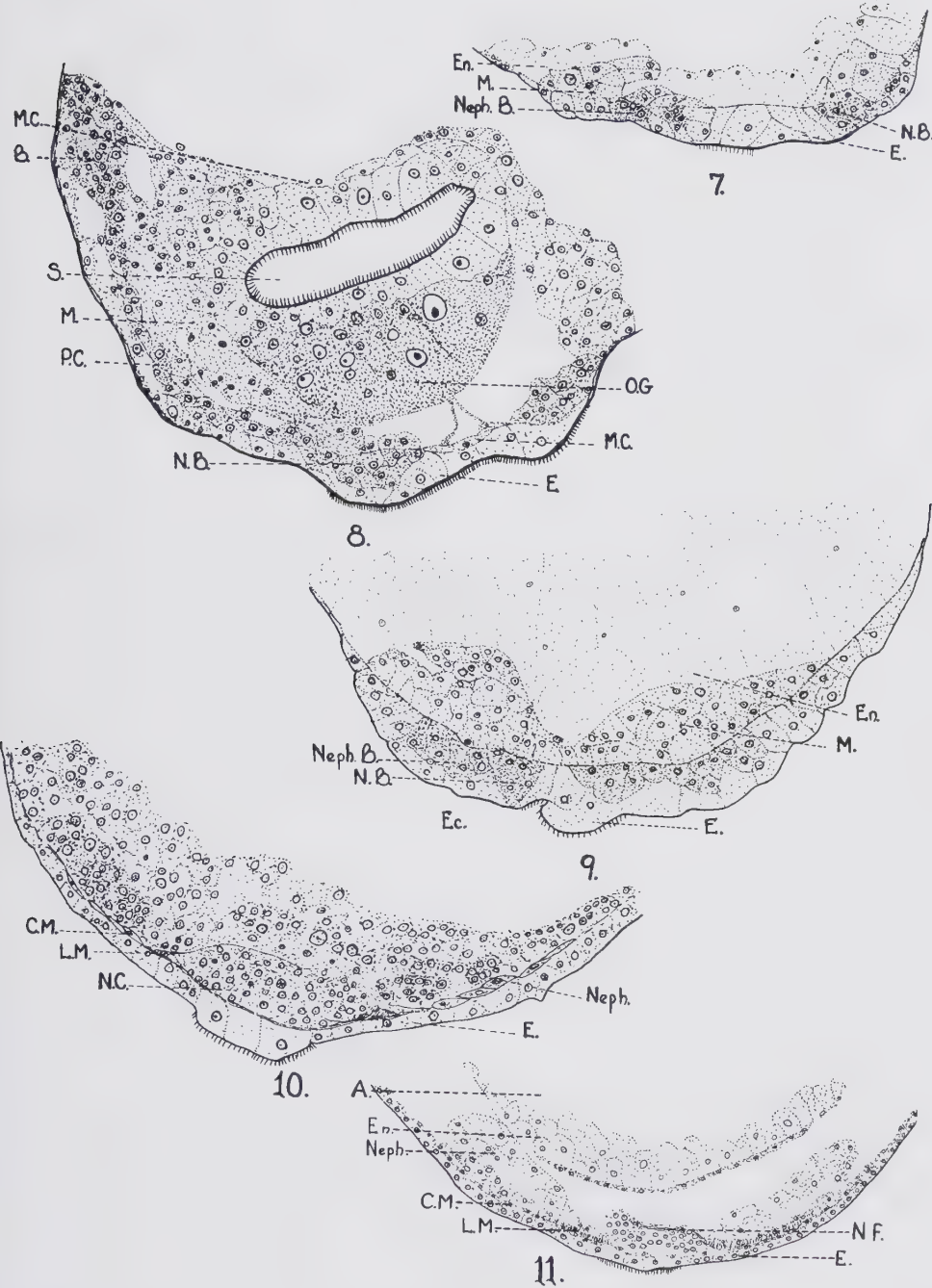


PLATE 3

EXPLANATION OF FIGURES

12 Stage of head flexion; showing lateral extension of longitudinal and circular muscles, fibers in ventral nerve cord, extension of lateral nerves, L.N., and a seta, S. $\times 165$.

13 Stage of weak crawling; section about one-half length of embryo, showing complete circular muscle layer, absence of longitudinal muscles on dorsal surface. $\times 263$.

14 Stage of normal crawling, showing completion of longitudinal and circular muscles and lateral nerves. $\times 263$.

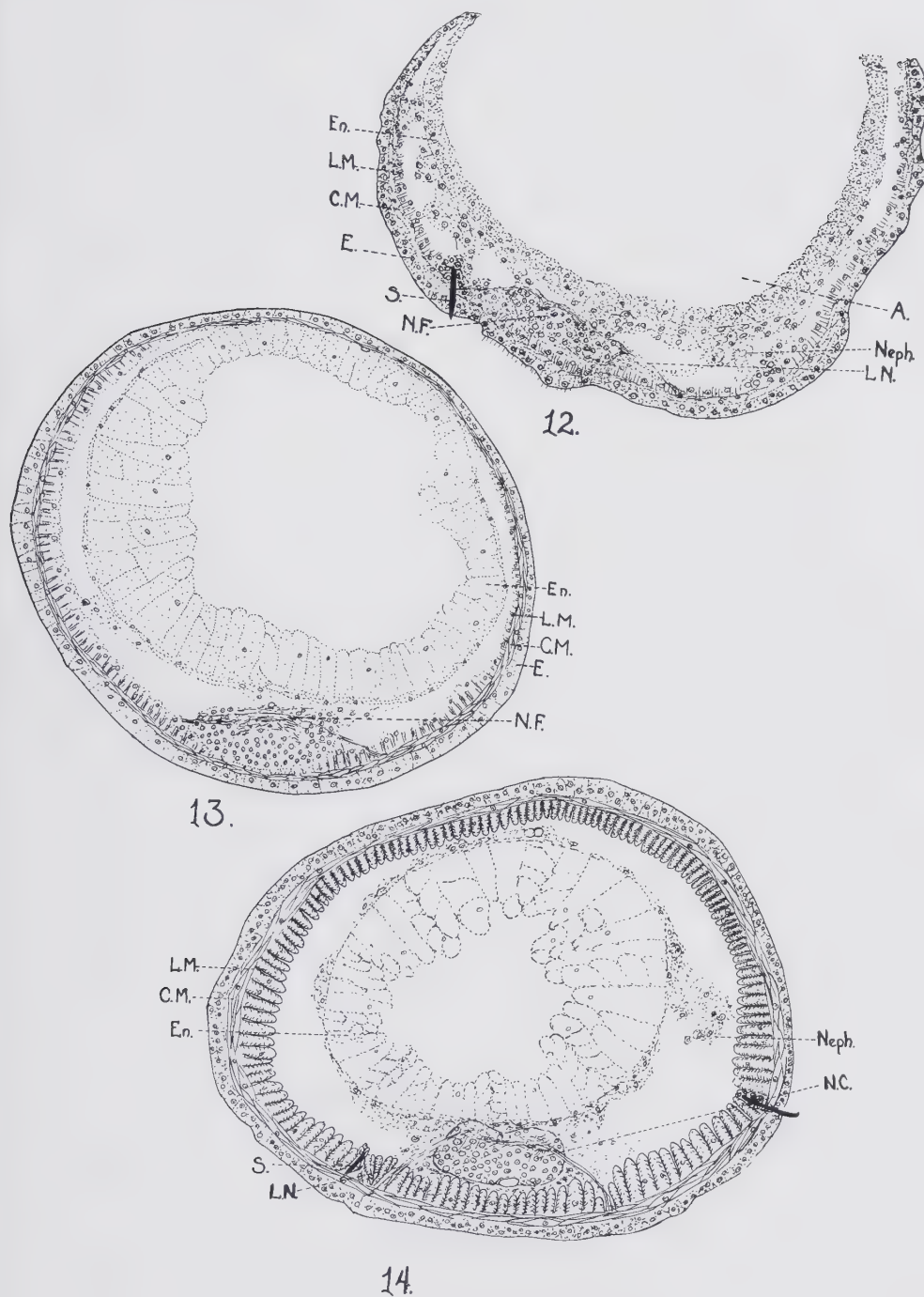


PLATE 4

EXPLANATION OF FIGURES

Photomicrographs of sagittal sections of anterior ends of embryos of *Eisenia*; fixation, Flemming or Gilson; stain, Heidenhain's haematoxylin and eosin.

15 Stage of stomodaeal contractions; showing stomodaeum, brain dorsal to it and oral gland ventral to it; few spindle-shaped muscle cells beneath pharyngeal epithelium. $\times 176$.

16 Stage of local surface contractions; showing brain, pharynx, oral gland, ventral cord, mesenchymatous cells around pharynx and albumen at its left in gut. $\times 176$.

17 Stage of head flexion; showing brain in first segment, beginning of both muscle layers, fibers in brain and in ventral nerve cord. $\times 85$.

18 Stage of weak crawling; showing brain in third segment, longitudinal and circular muscle layers, fibers in brain and ventral cord, and narrow area of albumen behind pharynx. $\times 85$.

19 Stage of normal crawling; showing sensory cells in epithelium, fibers in brain and ventral cord, prostomial nerve, beginning of pharyngeal pouch beneath brain. $\times 85$.

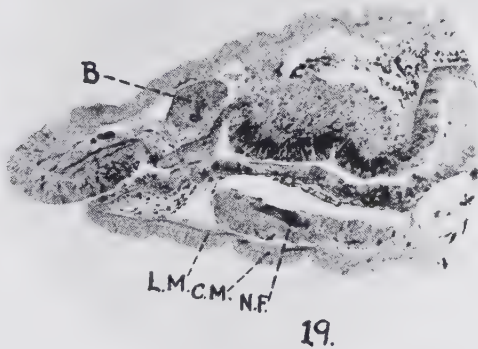
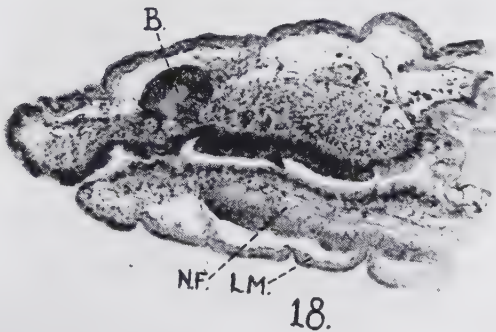
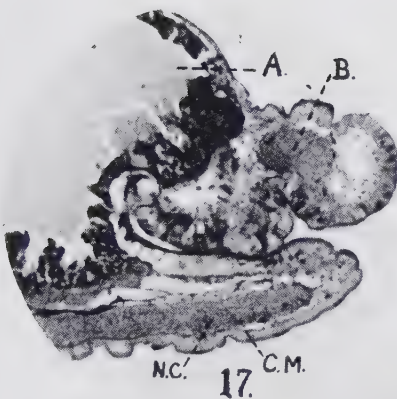
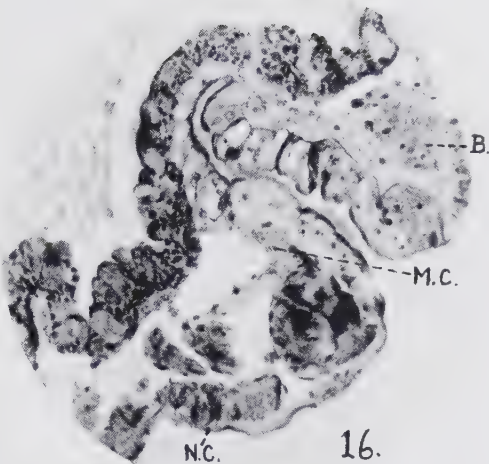
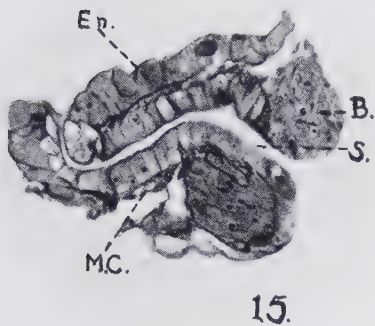


PLATE 5

EXPLANATION OF FIGURES

Schematic diagrams of embryos represented as dissected open dorsally and viscera removed, to show muscles and nerves. Heavy lines, lateral nerves; fine lines, muscles.

20 Stage of non-muscular activity; showing two neural bands two-thirds length of embryo and beginning of fusion of these bands above the mouth.

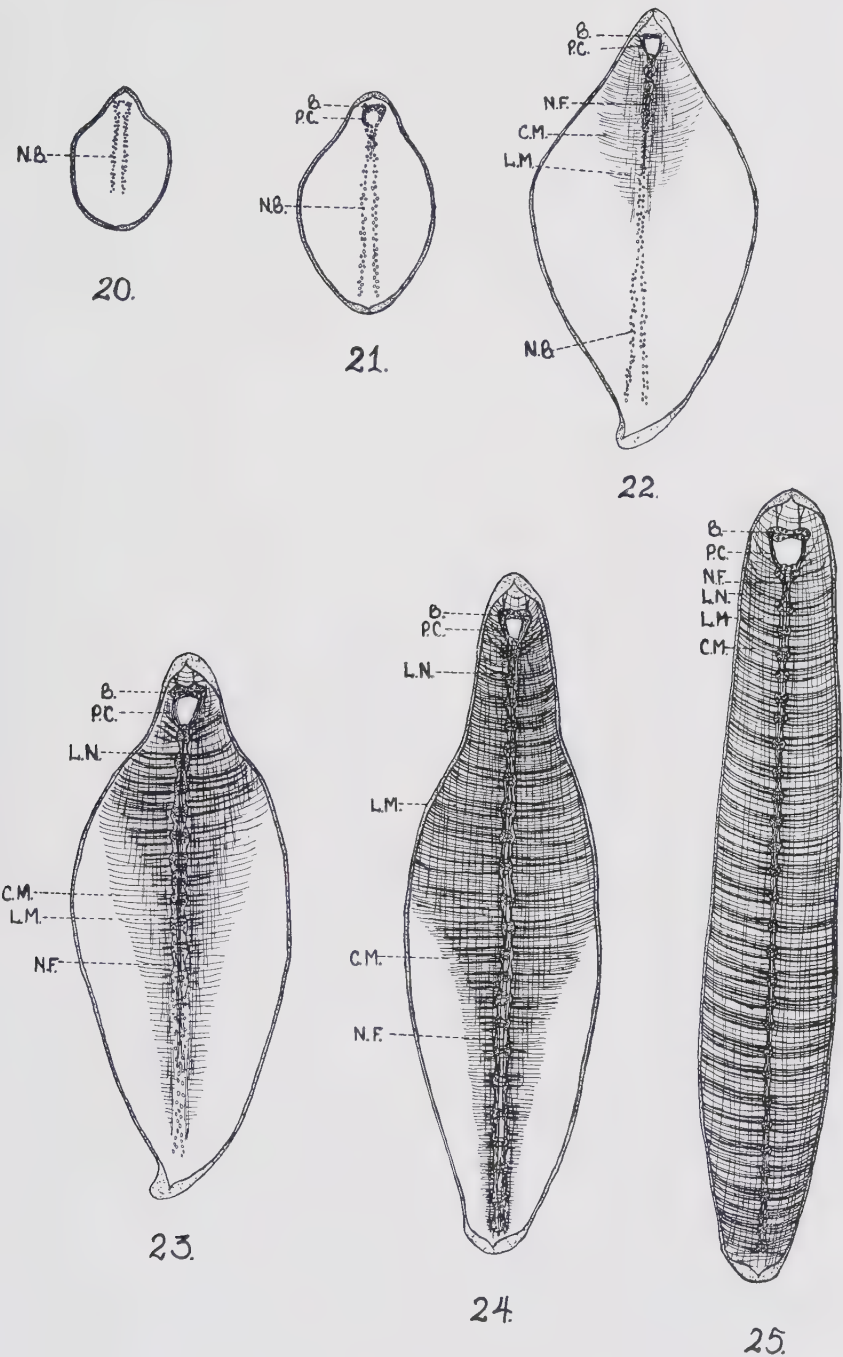
21 Stage of stomodaeal contraction; showing fusion of neural germ bands in brain and in first few ventral ganglia.

22 Stage of local surface contractions; showing fusion of neural bands two-thirds length, fibers in cord one-third length, longitudinal fibers slightly posterior to transverse ones, circular and longitudinal muscles in anterior third, longitudinal muscles slightly posterior to circular ones, the latter extending farther laterally.

23 Stage of head flexions; showing both muscle layers nearly to posterior end ventro-laterally and lateral nerves approximately two-thirds length.

24 Stage of weak crawling; showing extension of longitudinal and circular muscles and lateral nerves over all except posterior and dorsal surface.

25 Stage of normal crawling; showing completion of muscles and nerves over entire body.



EFFECT OF THE CENTRAL NERVOUS SYSTEM ON RESPONSES TO LIGHT IN EISENIA FOETIDA, SAV.

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ONE TEXT FIGURE AND TWO PLATES (ELEVEN FIGURES)

INTRODUCTION

Earthworms respond negatively in light of moderate and high intensities (Hoffmeister, 1845; Darwin, 1881; Graber, 1883; Loeb, 1894; Hesse, 1896; Parker and Arkin, '01; A. Smith, '02; Adams, '03; Holmes, '05; Harper, '05, '09; Mast, '11; Hess, '24, and Nomura, '26) and positively in light of very low intensities (Adams, '03; Hess, '24). Hesse and Hess found that the prostomium is most sensitive, segments I, II, and III less sensitive, the posterior segments still less and the middle of the body least sensitive to light. Graber, Loeb, and Hesse maintained that if the anterior segments are removed, earthworms continue to respond negatively, although less rapidly than normally. Hess found in *Lumbricus* that removal of the anterior three segments without removal of the brain causes a slight decrease in negative responses, but that removal of these segments with the brain causes a marked decrease in negative and corresponding increase in positive responses. He also found that removal of the brain without removal of the three segments, separation of the lobes of the brain and removal of one lobe have similar effects to that of removal of the brain with the three segments. Hess observed that, during the period of regeneration, the

¹It is a pleasure to acknowledge my indebtedness to Prof. S. O. Mast, who suggested the problem and directed the work, and also to Prof. E. A. Andrews for many valuable suggestions.

percentage of negative responses gradually returns to the normal value. Nomura ('26) removed different portions of the body from specimens of *Allolobophora* (*Eisenia*) and obtained results similar to those of Hess. He concluded that the brain causes negative and the ventral cord positive orientation.

These results indicate that there is an antagonistic action between the brain and the ventral nerve cord with respect to their function in the responses to light, but they have no precise bearing on the nature of this antagonistic action. The following pages contain the results of a study of this problem.

MATERIALS AND METHODS

Eisenia foetida, Sav.,² was used exclusively in this investigation. Part of the specimens were collected in a pile of decaying leaves and part in a compost pile. Those used during the winter were kept in large boxes filled with moist leaves in the laboratory and greenhouse. Before using them in experiments, the worms were kept at 20° to 21° for 24 hours or longer between pieces of moist paper toweling in glass finger bowls in a light-tight constant-temperature chamber. All of the specimens used were mature; they varied in length from 5 to 10 cm.

All the observations on the responses to light were made in a basement dark-room. The apparatus used consisted essentially of a 100-watt tungsten lamp in a dead-black box containing an opening 2 × 3 cm. on one side, and a horizontal glass plate 90 cm. from the lamp. Between the lamp and the glass plate were three screens. The first, immediately in front of the box, contained an opening 1.5 × 2.7 cm. which was covered by a shutter. The second, approximately 35 cm. in front of this, contained a frame into which a Wratten neutral tint filter or a square of plate glass could be inserted. The third, approximately 35 cm. in front of the second, con-

² *Enterion fetidum*, Savigny, 1862; *Allolobophora foetida*, Eisen, 1874; *Eisenia foetida*, Malm, 1877; *Helodrilus* (*Eisenia*) *foetidus*, Michaelsen, '10. (F. Smith, '17.)

tained an opening which reduced the width of the beam of light falling upon the glass to 3.8 cm. The glass plate was 16.5×21.5 cm. in size and over the center of it was suspended a needle used as a pointer. By varying the Wratten filters, light of 99, 9.9, 3.9 and 0.09 m.c. could be obtained on the glass plate beneath the pointer.

The temperature of the dark-room was kept between 20° and 22° , except on a few occasions when it went as high as 24° .

The observations were made as follows: The lamp in the box was turned on, the square of plate glass or a Wratten filter was put into the second screen, and the shutter in the first screen closed. A red lamp near the horizontal glass plate was now turned on, and all other lights in the dark-room were extinguished. A sheet of water-saturated black paper was put on the glass plate, then a worm was gently taken by means of a paraffined section lifter from the finger bowl which had been in the dark chamber and put on the glass plate. Almost invariably the worm immediately began to crawl forward. As soon as this occurred the plate was adjusted until the worm was moving perpendicular to the direction of the beam and directly toward the point under the pointer. When the anterior end reached this point, the shutter was opened. When illuminated in this manner the specimen either did not respond, or it stopped its forward locomotion; then it either contracted or turned the anterior end toward or from the light. If it turned, the direction of turning was recorded as positive or negative. If it contracted without turning or did not respond, no record was made. As soon as the response was obtained, the shutter was closed. The plate was then turned through 180° so as to expose the other side of the worm. The anterior end was now brought under the pointer, the shutter opened again and the response noted. This was repeated usually until ten responses were in hand, five to illumination from the right side and five to illumination from the left side. The whole process was then repeated with other individuals in the same or in different

luminous intensities until the desired number of records were obtained. Usually four or five specimens were tested in one intensity, then the same group was tested in another intensity in the same order. The whole process was later repeated with some individuals on which various operations had been made, with others which had been subjected to various temperatures, and with still others which had been injected with drugs. The results obtained are presented in the following sections.

RESPONSES TO LIGHT IN NORMAL EARTHWORMS

A summary of the results obtained in observations on the responses to lateral illumination in normal worms is pre-

TABLE 1

Responses of unoperated specimens of Eisenia to lateral illumination. Worms adapted at 20° to 21° for 24 hours before being tested.

Approximately 10 responses each

METER CANDLES	NUMBER OF SPECIMENS	PERCENTAGE NEGATIVE RESPONSES
0.09	74	10.7 ± 0.9
3.9	76	63.9 ± 1.9
9.9	114	92.6 ± 0.4
99.	100	98.6 ± 0.3
Average		71.5 ± 0.8

sented in table 1. This table shows that, in general, as the intensity increases, the percentage of negative responses increases and the percentage of positive responses decreases. The number of negative and positive responses is approximately the same irrespective of the side stimulated.

These results are in accord with the findings of Adams ('03) and Hess ('24), except that these authors did not find the worms to be predominantly positive until the intensity became much lower than 0.09 m.c. This may be explained in part by the fact established by Nomura and Ohfuchi ('28 a) that earthworms are more strongly negative to light in the spring and summer, when Hess' experiments were performed, than in the autumn and winter, when these experiments were performed.

It may be concluded, therefore, that the higher the luminous intensity, within certain limits, the more strongly negative are the worms and the lower the intensity, the more strongly positive they are.

RESPONSES TO LIGHT AFTER OPERATION ON THE CENTRAL
NERVOUS SYSTEM AND DURING THE PERIOD OF
REGENERATION FOLLOWING THE
OPERATION

The effect upon responses to light of several operations on the brain similar to those performed by Hess on *Lumbricus* was investigated. The operations were performed as follows: a specimen, anaesthetized in a saturated solution of chloretone, was put into water in a dissecting trough made of modeling clay; then, under a dissecting binocular, a longitudinal incision was made in the dorsal wall of the second and third segments, a needle inserted beneath the brain, and the desired cut made. The worm was then washed in tap water, put into a dark chamber at 20° to 21° and left for approximately 24 hours, after which the responses to light were ascertained as described above. The worm was now returned to the dark-chamber, after which its responses to light were ascertained once every 24 hours until it had completely regenerated. This was repeated with numerous groups of specimens except that some of them were fixed and sectioned before regeneration was complete and that in one specimen (a control) in each group an incision was made and a needle inserted beneath the brain but the nerve tissue not cut. Different operations were distributed at random among each series, and when testing the responses to light, the observer had little or no knowledge as to how a particular specimen had been treated.

The effect of the following operations was ascertained: 1) removal of the entire brain; 2) separation of the two lobes of the brain by a median cut; 3) removal of one lobe of the brain, and, 4) severance of one pharyngeal commissure. The specimens with operations 1 and 2 were tested in four intensities of light, those with operations 3 and 4 were tested in the two higher intensities.

The percentages of negative responses in a typical specimen before and after each operation are given in table 2. This table shows that, in general, each operation was followed by a decrease in the percentage of negative responses and that recovery of normal percentages of negative responses occurred after each operation. It also shows that after the entire brain or one lobe was removed the minimum percentages of negative responses were not reached until 2 to 4 days

TABLE 2
Effect of operations on responses to light in Eisenia. Records of typical specimens before and after operation

OPERATION	METER CAN- DLES	PERCENTAGE NEGATIVE RESPONSES												
		Before opera- tion	On successive days after operation											
			1	2	3	4	5	6	7	8	9	10	11	12
Brain removed	0.09	0	..	0	0	0	..	..	0	..	10	0	0	0
	3.9	60	..	20	37	10	40	10	10	20	30	20	30	60
	9.9	90	80	40	20	20	60	70	60	60	60	60	90	80
	99.	100	90	70	60	30	60	80	70	90	60	90	100	100
Lobes of brain separated	0.09	0	..	0	..	10	0	0	10	25				
	3.9	70	10	0	20	20	10	30	50	40				
	9.9	90	0	40	20	20	70	80	70	100				
	99.	90	20	50	80	80	100	80	90	100				
One lobe removed	9.9	90	90	60	50	50	50	50	50	80	90			
	99.	100	100	70	60	70	70	80	70	90	100			
One commissure cut	9.9	100	50	80	60	90								
	99.	100	60	90	100	100								

after the operation. This time, that is, the time between the operation and minimum negativity, is an injury shock period.

Recovery of normal negativity occurred in approximately the same time in the two lower intensities, 0.09 and 3.9 m.c., used in the first two operations, as in the higher intensities. No negative responses and few positive ones were obtained in 0.09 m.c. until regeneration was nearly complete.

A summary of the results obtained in the two higher intensities, 9.9 and 99 m.c., with all of the operated specimens is given in table 3. This table shows that the amount of decrease in percentage of negative responses after the different

operations is in the following order: lobes of brain separated > brain removed > one lobe removed > one commissure cut, and that the recovery period is in the following order: brain removed > one lobe removed > lobes separated > one commissure cut. The order of recovery is in accord with the amount of tissue which must be regenerated before the nervous structures involved in the response are restored. The order of the effect of the operations on the percentage of negative responses is not in accord with the amount of tissue lost. This

TABLE 3

Comparison of effects of four operations on responses to light in Eisenia

OPERATION	METER CANDLES	NUMBER SPECIMENS	MEAN OF MINIMUM PERCENTAGES NEGATIVE RESPONSES	PERCENTAGE OF CHANGE IN NEGATIVE RESPONSES	RECOVERY PERIOD, DAYS
Brain removed	9.9	9	25.5 ± 1.1	-71.8 ± 2.3	10.9
	99.	8	56.2 ± 3.8	-63.5 ± 3.2	10.2
	Average		30.8 ± 2.4	-67.6 ± 2.7	10.5 ± 0.5
Lobes of brain separated	9.9	13	15.7 ± 2.3	-82.5 ± 2.9	8.0
	99.	13	38.7 ± 2.5	-61.0 ± 2.4	6.8
	Average		26.9 ± 2.4	-71.7 ± 2.7	7.4 ± 0.3
One lobe removed	9.9	10	37.8 ± 3.6	-60.6 ± 2.9	9.3
	99.	10	53.1 ± 1.4	-46.9 ± 1.8	7.2
	Average		45.4 ± 2.5	-53.7 ± 2.3	8.2 ± 0.4
One commissure cut	9.9	13	44.7 ± 2.5	-53.0 ± 2.8	4.6
	99.	13	58.4 ± 2.9	-40.1 ± 2.1	4.2
	Average		51.5 ± 2.7	-46.5 ± 2.4	4.4 ± 0.2

order can be explained as follows: The time elapsing between operation and minimal negativity (the injury shock period) is longer after removal of the entire brain than after removal of one lobe and this period after both of these operations is longer than it is after the lobes of the brain are separated. As a result of this shock period the lowest percentage of negative responses is often not observed until the brain is well on the way in regeneration. Consequently, the longer the shock period, the more regeneration, and the less the apparent decrease in percentage of negative responses.

In a few specimens the ventral nerve cord was cut immediately behind the subpharyngeal ganglion. The results were substantially the same as those of Hess ('24). When laterally illuminated by 9.9 m.c. the anterior end turned from the source of illumination, and the region immediately behind the cut turned slowly toward it. This indicates that the ganglia behind the subpharyngeal ganglion induce positive responses.

Specimens in which the prostomial photoreceptors were eliminated showed little decrease in the percentage of negative responses. For example, one specimen in which both prostomial nerves were cut and the prostomium removed showed an average decrease of only 15 per cent in negative responses. Hess obtained similar results in *Lumbricus*. The effect of the operations is not, therefore, due to removal of the connections between the central nervous system and a large number of photoreceptors.

It was also found that in all the experiments, the control specimens in which the brain was uninjured, but in which a dorsal incision was made, responded nearly normally.

In addition to the decrease in percentage of negative responses resulting from interference with the brain by operation, it was noted that the general activity of the worms decreased during the injury shock period and then slowly increased during the recovery period. This inactivity was observed particularly with respect to spontaneous movements, the initiation of burrowing, and the time required to become light-adapted. The operated specimens became light-adapted sooner than unoperated specimens, that is, fewer consecutive responses could be obtained in a given intensity.

This evidence supports the conclusions of Hess and Nomura that, when the brain is removed, the body of an earthworm, under the influence of the ventral nerve cord, becomes more strongly positive in all intensities and that in intact worms the brain exerts some sort of influence which acts against the tendency of the ventral cord, resulting in negative responses. In general, it may be concluded that removal of

the entire brain or one lobe of the brain, separation of the two lobes or transection of one pharyngeal commissure increases the threshold intensity necessary for a preponderance of negative responses and that, therefore, it does not cause a true reversal in the sense of orientation.³

The question now arises as to which parts of the brain are necessary for the responses to light. A variety of results bearing on this problem will be presented in the following section.

LOCALIZATION OF FUNCTION IN THE BRAIN OF EISENIA

The problem concerning localization of function in the brain of *Eisenia* was attacked by study of the structure of normal brains, of regenerating brains, and of brains which had been injured locally. In all of these studies sections were made of the anterior ends of many worms. Some of these were fixed in Bouin's and stained with Mayer's haemalum or with Heidenhain's haematoxylin and eosin. Others were impregnated with silver nitrate. A number of silver nitrate techniques were tried, but the most favorable was Boulé's. Boulé's second fixative was found to be most satisfactory. This contains the following: 25 per cent formalin, 5 per cent acetic acid, and 0.5 per cent ammonium hydroxide. The specimens were impregnated with 3 per cent silver nitrate in 15 per cent alcohol and reduced in a mixture of 10 per cent formalin, 1 per cent hydroquinone, and 15 per cent alcohol. They were cleared in either bergamot or benzol.

Evidence from the structure of the brain

The studies of many histologists and the accompanying figures indicate the following: The brain of the earthworm consists of an outer layer of nerve cells and neuroglia and an inner core of nerve fibers, the neuropile (fig. 2). Most

³ The fact that Graber, Loeb, and Hesse obtained negative responses in brainless worms was doubtless because they used high intensities; these results are in accord with those of Hess and Nomura, who controlled the intensity of light more carefully.

of the cell bodies in the brain are on the dorsal surface in contrast with their location in the nerve cord where most of them are ventral. In the neuropile, the fibers are exceedingly abundant, fine in structure, and densely crowded together, and some are grouped in dorsal and ventral strands of transverse fibers. These fibers of the transverse commissure originate in the peripheral cells, in the roots of the prostomial nerves and in the roots of the pharyngeal commissure.

The prostomial nerves emerge at the antero-ventral border of the brain and innervate the prostomium and the buccal cavity. These nerves are almost entirely sensory and receive impulses from chemical, moisture, tactile, and photic sense-organs. Horizontal sections and sagittal sections at the point of entrance of a prostomial nerve (fig. 3) show that the roots of these nerves diffuse widely in the anterior and the posterior regions as well as in the dorsal and the ventral parts of the brain. Similarly, cross sections at the union of the pharyngeal commissures with the brain (figs. 1 and 12) show that fibers enter each commissure from the dorsal and the ventral parts of the neuropile of the brain. Sagittal sections (fig. 2) show that the same holds for anterior and posterior fibers which contribute to the commissure.

The following conclusions can be drawn on the basis of this evidence: 1) The fibers of both prostomial nerves and pharyngeal commissures diffuse widely posteriorly, anteriorly, dorsally, and ventrally. Hence little localization can be expected in the roots of these nerves. 2) The fibers in the neuropile of the brain are so numerous and so crowded that it is impossible to trace single fibers for any considerable distance or to state the location of the cell bodies connected with the fibers in various parts of the brain. Experimental methods must, therefore, be employed in the investigation of the course of various fibers in the brain.

Evidence from regenerating specimens

It was shown above (table 2) that normal negativity to light usually returns gradually during the process of regen-

eration. Specimens which had reached different percentages of negativity during recovery were fixed and sectioned. The sections were studied with the view to ascertaining the relation between the structure of the brain in different stages of regeneration and the character of the responses to light.

The transverse commissure in the brain of approximately twenty-five specimens was transected at different times, either by a complete separation of the two lobes of the brain or by severing the connection between the lobes except for the brain sheath on one side. In all of these specimens the percentage of negative responses was greatly decreased as a result of the operation. Ten of these were fixed on the day after operation. The same general structure was found to be present in all of them. Figure 4 shows that the space between the lobes of the brain was completely filled with loose cells, the cicatrix, but that no fibers connected the two halves of the brain.

Specimens fixed several days after this operation show that nerve fibers were beginning to extend from the cut surfaces into the cicatrix. The time of the first contact of neurones between the two lobes is difficult to ascertain, but figure 5 illustrates the condition shortly after the first strand of fibers extends from one lobe to the other. These specimens, on the day they were fixed, had increased markedly in negativity. Sections of three specimens in which the lobes had been separated but in which negative responses had returned to normal showed that there were fewer than the normal number of fibers between the lobes.

Sections were prepared of seven of the specimens from which the entire brain was removed. Two fixed on the day following removal of the brain showed that the region between the cut ends of the commissure was filled with cicatrix. Sections of one of the five which had completely recovered in negative responses before fixation (fig. 6) show that the width of the neuropile in the brain was less than in the subpharyngeal ganglion and that the width of the center of the neuropile of a normal worm (fig. 2) is approximately twice that in this specimen.

Sections of five of the ten specimens from which one lobe of the brain was removed were prepared after the specimens had recovered normal negativity. In one of these specimens the regenerated lobe contained nearly as many fibers as the normal lobe, but in the other four specimens it contained many fewer fibers (fig. 7).

One of the specimens in which one pharyngeal commissure had been cut was fixed before complete recovery occurred. This specimen (fig. 8) shows that new fibers were extending from the dorsal or brain surface of the cut toward the commissural surface. Four specimens, fixed as soon as they had recovered normal negativity, showed that a few fibers extended across the wound but that not nearly all of the fibers had regenerated.

The evidence presented from a study of specimens undergoing regeneration after each of the four operations agrees in that in all specimens which were sectioned immediately after complete recovery of normal negativity, fewer than the normal number of fibers were present. Although relatively few specimens were sectioned immediately after complete recovery, these indicate that the worms are not normally negative when only a very few fibers extend across the wound, but that not all of the fibers are required for normal negativity. Sudden rapid increases in negativity during recovery appear to be correlated with the first contact of fibers across the wound and subsequently with contact of additional groups of fibers. Hence a quantitative factor seems to be involved in that a certain number of fibers seem to be necessary for a certain percentage of negative responses. This necessity for a slight connection between the lobes of the brain, the fact that practically the same effect is obtained when the brain is split as when it is entirely removed, and the diffuse nature of the roots of the prostomial nerves and the pharyngeal commissures, all indicate that the transverse commissure is the essential region involved in this response. The significance of the location of the fibers in this transverse commissure will be considered in the following section.

Evidence from local cuts and cauterizations in the transverse commissure of the brain

Specimens which were to be used in these experiments were kept in a dark chamber at 20° to 21° for 24 hours and at the end of that time their responses to light were ascertained. They were then anaesthetized in a solution of chloretone, a dorsal incision made in the third segment, and then, by means of a needle which had been ground to a cutting edge, an incision was made in some part of the transverse commissure of the brain. The worms were then kept in the dark for approximately 20 hours, after which their responses to light were again ascertained. They were now returned to the dark chamber, left 8 hours, after which their responses were again ascertained. The specimens were then fixed and sectioned and the actual extent of the injury ascertained. Forty-four specimens were operated upon in this manner. The same method was used with three others except that instead of being cut, the transverse commissure of the brain was cauterized locally.

The results obtained in observations on thirteen typical specimens of the forty-seven specimens studied are presented in table 4. This table shows the following: After anterior, posterior, dorsal or ventral incision of the brain, twenty of the forty-seven specimens showed a decrease in percentage of negative responses practically as great as that produced by removal of the brain. No transverse fibers connected the two lobes of the brain in any of these. Twenty-three of the specimens remained nearly normal in percentage of negative responses. One of these had no fibers in the transverse commissure, an anomalous case; nine had at least half of the fibers in the transverse commissure, and thirteen had less than half. In those with less than half of the fibers it made no difference whether the fibers present were in the posterior (fig. 9), in the anterior (fig. 10) in the dorsal (fig. 11), or in the ventral part (fig. 12) of the commissure. Four specimens, one with an anterior incision, one with a posterior incision, and two with dorsal incisions had only one-eighth to

one-quarter the normal number of fibers intact. In all of these specimens the decrease in percentage of negative re-

TABLE 4

Effect on responses to light of cutting or cauterizing portions of the transverse commissure of the brain

PERCENTAGE OF NEGATIVE RESPONSES		AVERAGE PER CENT CHANGE IN NEGATIVE RESPONSES	CONDITION OF BRAIN	NUMBER OF SIMILAR SPECIMENS
9.9 meter candles	99 meter candles			
Anterior incision				
37	68	—45	Transverse commissure transected	2
95	95	— 5	Nearly perfect brain	2
90	90	—10	Few fibers just posterior to middle of brain	3
Posterior incision				
20	45	—62	Lobes connected by sheath, but no fibers between them	3
100	100	+10	Nearly normal brain	3
100	100	— 5	Less than half of anterior trans- verse fibers present	5
Dorsal incision				
10	30	—69	No continuous fibers in transverse commissure	8
100	100	+10	Nearly perfect brain	2
93	95	— 4	Narrow band of ventral transverse fibers	4
Ventral incision				
14	50	—66	Lobes of brain completely sepa- rated, dorsal sheath continuous	4
90	100	— 5	Brain nearly normal	2
90	100	0	Few dorsal transverse fibers present	4
Deep incision				
65	74	—30	Very thin strand of antero-dorsal fibers in transverse commissure	4

sponses was approximately half as great as it was in specimens in which all the fibers had been cut but much greater than in specimens in which half the fibers remained intact, no matter in which region they were.

These facts support the conclusions reached in the preceding two sections, namely, that there is localization of function in the brain of *Eisenia*, the transverse commissure being necessary for normal percentages of negative responses to light, that there is no gross localization of function within this commissure, and that there is a roughly quantitative relation between the number of functional fibers and the character of the response, normal percentages of negative responses requiring the presence of less than half the fibers in the commissure.

NATURE OF THE INTERACTION BETWEEN BRAIN AND VENTRAL NERVE CORD

In the preceding pages it was demonstrated that there is an antagonistic action between the brain and the ventral nerve cord of *Eisenia* in the response to light and that the transverse commissure of the brain is essential for normal responses. The manner of action of this commissure in the antagonistic action between brain and cord remains to be considered. Direct evidence from the effects of operation will be presented concerning the function of the transverse fibers and indirect evidence from the effect of different temperatures and drugs on the brain as a whole.

The function of the transverse commissure

The number of positive and negative responses in specimens with the brain removed or split or with one lobe removed is essentially the same irrespective of the side illuminated. But the following evidence shows that this does not obtain in specimens with one pharyngeal commissure cut. In seven specimens the right pharyngeal commissure was transected. Twenty-four hours after the operation these specimens gave a total of thirty-two negative and three positive responses to illumination (9.9 m.c.) on the right and six negative and twenty-nine positive responses to illumination on the left. Six specimens in which the left commissure was cut gave

twenty-two negative and one positive responses to illumination on the left and six negative and fourteen positive responses to illumination on the right. Obviously, then, the majority of positive responses were obtained in illumination of the unoperated side. Moreover, there was a tendency to turn the head toward the unoperated side irrespective of the side illuminated. Hess observed the same tendency in *Lumbricus*, but offered no explanation.

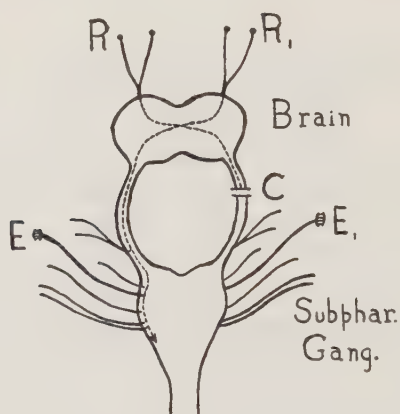


Fig. 1 Diagram illustrating crossing-over of tracts in brain and effect of transection of one pharyngeal commissure. R and R₁, prostomial photoreceptors; E and E₁, muscles of body; C, cut in right pharyngeal commissure; Subphar. Gang., subpharyngeal ganglion.

This effect of cutting one pharyngeal commissure can be explained by assuming that the fibers connected with the receptors on the right side of the anterior end cross in the transverse commissure of the brain to the left lobe and vice versa, as illustrated in figure 1. This figure indicates that impulses received at the receptors R₁ on the right side of the prostomium pass to the left side of the brain and cord and induce the muscles on the left side to contract, causing the anterior end to turn away from the side illuminated. It also indicates that in case the right commissure is cut an impulse

from the left side crosses in the brain but is stopped by the cut, and that without this influence of the brain the effect of the cord is predominant and the worm turns toward the light. This assumption is strongly supported by the preceding evidence that the transverse commissure in the brain contains the fibers which are involved in the negative responses to light.

Effect of temperature on responses to light in Eisenia

It is well known that nervous activity and the nature of responses to light are usually specifically correlated with temperature. Nomura and Ohfuchi ('26) maintained that heat increases with negativity to light in *Eisenia*.

Observations on the effect of temperature on responses to light were made as follows: An unoperated worm was kept in darkness at 20° to 21° for 24 hours; then its responses in lateral illumination were ascertained at this temperature. It was then returned to darkness at a different temperature and left for 24 hours, after which it was returned to room temperature (20° to 21°) and its responses again ascertained. Frequently two tests were made with a few hours intervening between them. This procedure was repeated with other normal worms and with specimens in which the brain was split before subjection to altered temperature. The results of these experiments are summarized in table 5.

This table shows the following: Unoperated specimens which had been subjected to low temperatures were, in all intensities tested, less negative than those kept at room temperature and those subjected to high temperatures were more negative. In general, as the temperature rose, the percentage of negative responses increased to a maximum at 25° to 26° and then decreased slightly. Operated specimens which had been subjected to low temperatures were less strongly negative to light than those which had been subjected to high temperatures; that is, as the temperature increased from 10° to 30° the effect of the operation diminished.

It was also noted that worms subjected to low temperatures were very sluggish while those which had been subjected to 25° and 30° were more active than normally. This is in accord

TABLE 5

Effect of subjection for 24 hours to different temperatures on responses to light in Eisenia. Operated specimens, worms in which the lobes of the brain had been separated by a median cut

TEMPERATURE	METER CANDLES	UNOPERATED SPECIMENS			OPERATED SPECIMENS		
		Number specimens	Percentage negative responses	Per cent change in negative responses	Number specimens	Percentage negative responses	Per cent change in negative responses
10-11°	0.09	8	No responses in 6 Positive responses in 2		6	No responses in 5 Positive responses in 1	
10-11°	3.9	8	33.6±1.4	— 43.4±1.2	7	11.7±2.2	—81.0±3.8
10-11°	9.9	8	51.1±2.6	— 37.2±3.4	7	17.7±3.0	—81.1±3.0
10-11°	99.	8	77.7±3.5	— 21.2±3.8	7	32.7±3.2	—67.2±2.9
10-11°	Avg.		40.6±1.9	— 32.9±2.8		15.5±2.1	—76.2±3.2
15-16°	0.09	5	4.6±1.9	— 8.6±1.1			
15-16°	3.9	7	47.4±2.8	— 23.0±2.2			
15-16°	9.9	7	76.5±4.1	— 22.3±4.3			
15-16°	99.	7	91.4±2.5	— 7.2±2.2			
15-16°	Avg.		54.9±2.8	— 29.4±2.9			
20-21°	0.09	22	10.6±0.7		12	2.7±0.9	—78.2±2.0
20-21°	3.9	82	68.3±1.2		12	19.0±4.2	—64.3±4.2
20-21°	9.9	100	91.9±0.3		13	25.6±4.5	—71.0±5.1
20-21°	99.	100	98.7±0.3		13	53.3±3.7	—46.5±4.1
20-21°	Avg.		67.4±0.6			25.1±3.3	—65.0±3.8
25-26°	0.09	7	24.2±3.8	+ 332.1±6.0			
25-26°	3.9	8	80.3±0.7	+ 24.8±1.6			
25-26°	9.9	8	93.7±1.6	+ 4.8±3.2			
25-26°	99.	8	98.0±1.0				
25-26°	Avg.		74.0±1.8	+ 120.5±3.6			
30-31°	0.09	9	31.4±2.0	+ 159.5±3.3	6	3.3±1.2	—64.5±1.2
30-31°	3.9	10	70.0±3.1	+ 22.5±2.1	6	43.6±2.0	—30.1±1.5
30-31°	9.9	9	99.0±0.6	+ 2.2±1.3	6	63.8±2.8	—29.0±5.1
30-31°	99.				6	72.3±2.3	—27.6±2.3
30-31°	Avg.		74.0±1.9	+ 61.4±2.2		45.6±2.0	—37.8±2.9

with the results of A. Smith ('02), who found abnormal movement above 30° and death at 35°.

From these observations it is evident that low temperatures tend to have the same sort of effect as interference with the functioning of the brain and low intensities of light

in that they decrease the negativity to light and also general activity. High temperatures, on the contrary, tend to have the opposite effect and, like an intact brain and high intensities of light, increase the negative responses.

Effect of drugs on responses to light in Eisenia

Moore ('21) found that certain salts and excitant drugs, for example strychnine, stimulate the central nervous system of *Lumbricus*. Nomura ('27) affirmed that some drugs, for example strychnine nitrate, increase and that others, for example KCN and strychnine, decrease negative responses to light in *Eisenia* through their action on the brain and the ventral nerve cord. Nomura and Ohfuchi ('28 and '30) maintained that some inorganic salts act similarly.

In the investigation of the effects on photic response of several nerve-depressant and nerve-excitant drugs, approximately 0.05 to 0.1 cc. of a solution of the drug under consideration was, by means of a hypodermic needle, injected into the coelomic cavity above the brain of a worm which had been in darkness at 20° to 21° for 24 hours. The worm was then returned to darkness and left 1 hour, after which its responses to lateral illumination were ascertained. After 2 hours more in darkness its responses were again ascertained; and again after 9 hours more in darkness, that is, 12 hours after the injection. In this way there was ascertained the effect of benzyl alcohol 3.5 per cent, nicotine tartrate 1 per cent, nicotine 1 per cent, 0.1 per cent, and 0.01 per cent, strychnine sulphate 0.1 per cent, and cocain HCl 1.0 per cent and 0.1 per cent.⁴ Specimens injected with water were used as controls.

The results obtained 1 hour after injection with all the drugs except pure nicotine are presented in table 6. This

⁴ The drugs were obtained through the kindness of Dr. David I. Macht, lecturer in pharmacology, The Johns Hopkins University, and director, Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore. The author is also indebted to Doctor Macht for valuable suggestions concerning the investigation of the action of drugs.

table shows that in all of the intensities of light tested, the depressant drugs decreased the percentage of negative responses to light, that the excitant drugs increased them and that the injection of water had no effect. The percentages of negative responses 3 hours after injection were approxi-

TABLE 6

Effect of drugs on responses to light in Eisenia. Drugs injected dorsally into third segment. Responses 1 hour after injection

NERVE DEPRESSANT DRUGS			
METER CANDLES	Drug	Percentage negative responses	Per cent change in negative responses
0.09	Benzyl		
3.9	alcohol	32.0 ± 3.6	— 49.9
9.9	3.5 per cent,	43.4 ± 0.7	— 50.1
99.	10 specimens	56.8 ± 4.5	— 41.4
Average		44.0 ± 2.9	— 47.4 ± 2.8
0.09	Nicotine		
3.9	tartrate	17.5 (4 specimens)	— 72.6
9.9	1 per cent,	34.1 ± 3.3	— 63.1
99.	10 specimens	52.1 ± 4.5	— 47.0
Average		34.4 ± 3.9	— 60.9 ± 3.9
0.09	Cocain		
3.9	HCl	27.3 ± 2.2	— 57.2
9.9	1 per cent,	39.5 ± 2.5	— 57.3
99.	7 specimens	51.2 ± 2.6	— 47.1
Average		42.0 ± 2.4	— 53.8 ± 2.4
NERVE EXCITANT DRUGS; CONTROL			
0.09	Strychnine	51.3 ± 2.3	+ 370.0
3.9	sulphate	80.2 ± 1.0	+ 36.4
9.9	0.1 per cent,	95.0 ± 1.4	+ 2.5
99.	10 specimens		
Average		75.5 ± 2.3	+ 136.3 ± 1.6
0.09	Cocain	52.0 ± 1.1	+ 385.9
3.9	HCl	82.1 ± 1.1	+ 28.3
9.9	0.1 per cent,	98.0 ± 0.8	+ 5.6
99.	11 specimens		
Average		78.0 ± 1.1	+ 139.9 ± 1.1
0.09	Water	10.0	— 7.0
3.9	5 specimens	68.2	+ 6.9
9.9		95.0	+ 3.6
99.		96.4	— 2.2
Average		67.5	— 0.2

mately 10 per cent higher for the depressant drugs and 10 per cent lower for the excitant drugs than they were 1 hour after injection. The control specimens, injected with water, decreased very slightly, if at all, in negative responses during the first hour and were normal thereafter.

A few specimens were injected with pure nicotine. Nine of these were injected with 1 per cent nicotine; only two of these lived and they gave a majority of positive responses in the higher intensities 21 hours after injection. Four were injected with 0.1 per cent nicotine; two of these lived; in these the negative responses decreased approximately 75 per cent. Three specimens were injected with 0.01 per cent nicotine; in these the percentage of negative responses decreased slightly more than in the specimens which had been injected with a solution of 1 per cent nicotine tartrate. These results indicate that the effect of pure nicotine in a given concentration is much greater than that of nicotine of the same concentration in nicotine tartrate.

In addition to this marked decrease in percentage of negative responses resulting from injection of the depressant drugs, the general activity of the worms was considerably depressed. Strychnine seemed to increase activity, but cocain had only a very slight effect, if any.

A few specimens were injected at various points along the ventral nerve cord with benzyl alcohol and nicotine tartrate. The segments injected were paralyzed. The worms behaved in a manner similar to those with the ventral nerve cord cut posterior to the subpharyngeal ganglion. Spontaneous crawling was lacking. The anterior segments turned away from the light with about the normal negative percentages, but the body did not follow. This shows that the effect of the injection of these drugs in the dorsal part of the coelomic cavity of the third segment as given in table 6 was due to their action on the brain, rather than to their action on the rest of the nervous system owing to diffusion.

It may be concluded, therefore, that the nerve depressant drugs, nicotine tartrate 1 per cent, nicotine 0.01 per cent,

cocain HCl 1 per cent, and benzyl alcohol 3.5 per cent, when injected dorsally into the third segment of *Eisenia*, act much like low temperatures and operations on the brain in that they decrease the percentage of negative responses to light; and that the nerve excitant drugs, strychnine sulphate 1 per cent and cocain HCl 0.1 per cent, act like high temperatures in that they increase the percentage of negative responses to light. Probably the action of the inorganic salts described by Nomura and Ohfuchi ('28 and '30) is similar to that of these drugs.

DISCUSSION

The evidence presented above demonstrates that low intensities of light, interference with the function of the brain by operation, low temperatures, or nerve depressant drugs tend to decrease the percentage of negative responses to light and that high intensities of light, intact brain, high temperatures, or nerve excitant drugs tend to increase it. It also indicates that, in general, those factors which decrease the negativity to light decrease the general activity of the worm. It may be concluded from this evidence that decrease in percentage of negative responses is correlated with decrease in activity of the worm or of some part of it. From the effects of the drugs and operations it may be concluded that this decrease is in some way associated with the brain, and the evidence from various operations points to the further conclusion that the decrease is correlated with the transverse commissure. That is, it is concluded that low activity associated with the transverse commissure is correlated with low percentages of negative responses, and that those factors which increase the percentage of negative responses do so by accelerating activity of the transverse commissure and that those factors which decrease it do so by diminishing the activity of these transverse tracts.

The evidence presented from the effect of cutting one pharyngeal commissure also indicates that impulses produced by illumination on one side of the anterior end cross to the opposite side and cause contraction on this side, resulting in

negative responses. It may be concluded from this that the impulses cross from one side to the other by means of the transverse commissure. This conclusion, as well as the previous one, is supported by the evidence from a study of the structure of normal brains, regenerating brains, and locally injured brains, which indicates that a certain number of the fibers in the transverse commissure are necessary for normal responses to light.

If these conclusions are valid, the interaction between the brain and the ventral cord in response to light probably occurs somewhat as follows: Photic stimuli set up impulses in receptors throughout the body, particularly the prostomium. These impulses proceed to the central nervous system, some of them go to the cord where impulses are set up which tend to induce the muscles on the side stimulated to contract; others pass to the brain where they are probably modified, amplified, or set up new impulses and then cross over to the opposite side, pass through the pharyngeal commissure and the cord and out to the muscles on the opposite side. These impulses are stronger than those already present in the cord and consequently the muscles on the side away from the stimulating agent contract, causing the earthworm to turn away from the light. When, under certain conditions, the activity of the brain decreases or is absent entirely, the stronger impulses from the brain no longer oppose the impulses from the opposite side of the cord, cephalic dominance is lacking, and the worm turns toward the light.

SUMMARY

1. Normal specimens of *Eisenia foetida* respond negatively to high and moderate intensities and positively to low intensities of light.

2. Marked decrease in the percentage of negative responses to light results from: removal of the brain, separation of the two lobes of the brain by a median cut, removal of one lobe of the brain, transection of one pharyngeal commissure, subjection to subnormal temperatures, and injection over the

brain of nerve depressant drugs, benzyl alcohol 3.5 per cent, nicotine 0.01 per cent, nicotine tartrate 1 per cent, and cocain HCl 1 per cent.

3. An increase in percentage of negative responses to light results from: intact brain, subjection to supranormal temperatures, and injection over the brain of nerve excitant drugs, strychnine sulphate 0.1 per cent, and cocain HCl 0.1 per cent.

4. As the temperature to which worms have been subjected rises from 10°, the percentage of negative responses increases to a maximum at 25° to 26° and then diminishes slightly. Exposure to low temperatures increases and exposure to high temperatures decreases the effect of separation of the lobes of the brain. Low temperatures decrease and high temperatures increase general activity of *Eisenia*.

5. Recovery from the effect of both depressant and excitant drugs in the concentrations used (except nicotine) is nearly complete 12 hours after injection.

6. Operations on the brain are followed by a temporary period of injury shock after which the percentage of negative responses decreases to a minimum; this is followed, during regeneration, by a period of recovery of normal negative responses. The length of shock period and recovery period vary with the severity of the operation.

7. In addition to decreasing negativity to light, operations on the brain increase the ease with which these earthworms become light-adapted and diminish the general activity of the organism.

8. Evidence from the histology of normal brains, regenerating brains, brains in which the transverse commissure was locally cut or cauterized, indicates that normal negative responses persist during extensive brain injuries provided that not all the fibers in the transverse commissure between the lobes of the brain are destroyed. There is no evidence of strict localization of function in this commissure, but apparently a definite number of fibers is necessary for a certain degree of negativity.

9. Transection of one pharyngeal commissure is followed by decrease in negative responses to illumination on the normal side, but not to illumination on the operated side. This indicates that the impulses involved in this response cross over from one side of the brain to the other.

10. It is concluded that the normal cephalic dominance in the response to light in *Eisenia* can be explained on the assumption of a dorsal crossing-over of the functional tracts concerned and the assumption that diminution of activity of the brain or removal of the active regions in the brain eliminates the cephalic impulses which normally oppose those from the ventral cord resulting in positive responses.

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PLATES

ABBREVIATIONS USED IN FIGURES

Sagittal, horizontal and cross sections of brains of *Eisenia foetida*. D, dorsal; V, ventral; A, anterior; P, posterior; B, brain; C, cicatrix. Drawings by aid of camera lucida, slightly schematized; stipple, wound tissue in operated specimens; fine lines and stipple, nerve fibers; circles and dots, neuroglia and nerve cells.

PLATE 1

EXPLANATION OF FIGURES

Drawings of normal and regenerating brains.

2 Cross section of normal brain showing relative thickness of cellular layer and neuropile, dorsal and ventral groups of transverse fibers and fibers from both dorsal and ventral parts of the brain contributing to the pharyngeal commissures. Silver impregnation. $\times 143$.

3 Sagittal section showing entrance to brain of prostomial nerve and pharyngeal commissure, fibers from prostomial nerve passing to dorsal and ventral, anterior and posterior parts of the brain and fibers from anterior and posterior parts of the brain contributing to the pharyngeal commissure. Silver impregnation. $\times 143$.

4 Cross section of brain of specimen fixed on day following separation of the two lobes of the brain, showing wound tissue completely filling region between the lobes. This specimen was 43 per cent more positive than before operation. Silver impregnation. $\times 143$.

5 Cross section of brain showing beginning of regeneration after lobes had been separated, and showing few nerve fibers between the two lobes. This specimen was 21 per cent more negative than its lowest percentage of negative responses. Haematoxylin. $\times 143$.

6 Oblique cross section of a regenerating brain, the cut of the operation having been made at about the middle of the pharyngeal commissures. Compare width of neuropile of regenerated brain with that of normal brain and with that of subpharyngeal ganglion. This specimen gave normal negative responses. Haematoxylin. $\times 110$.

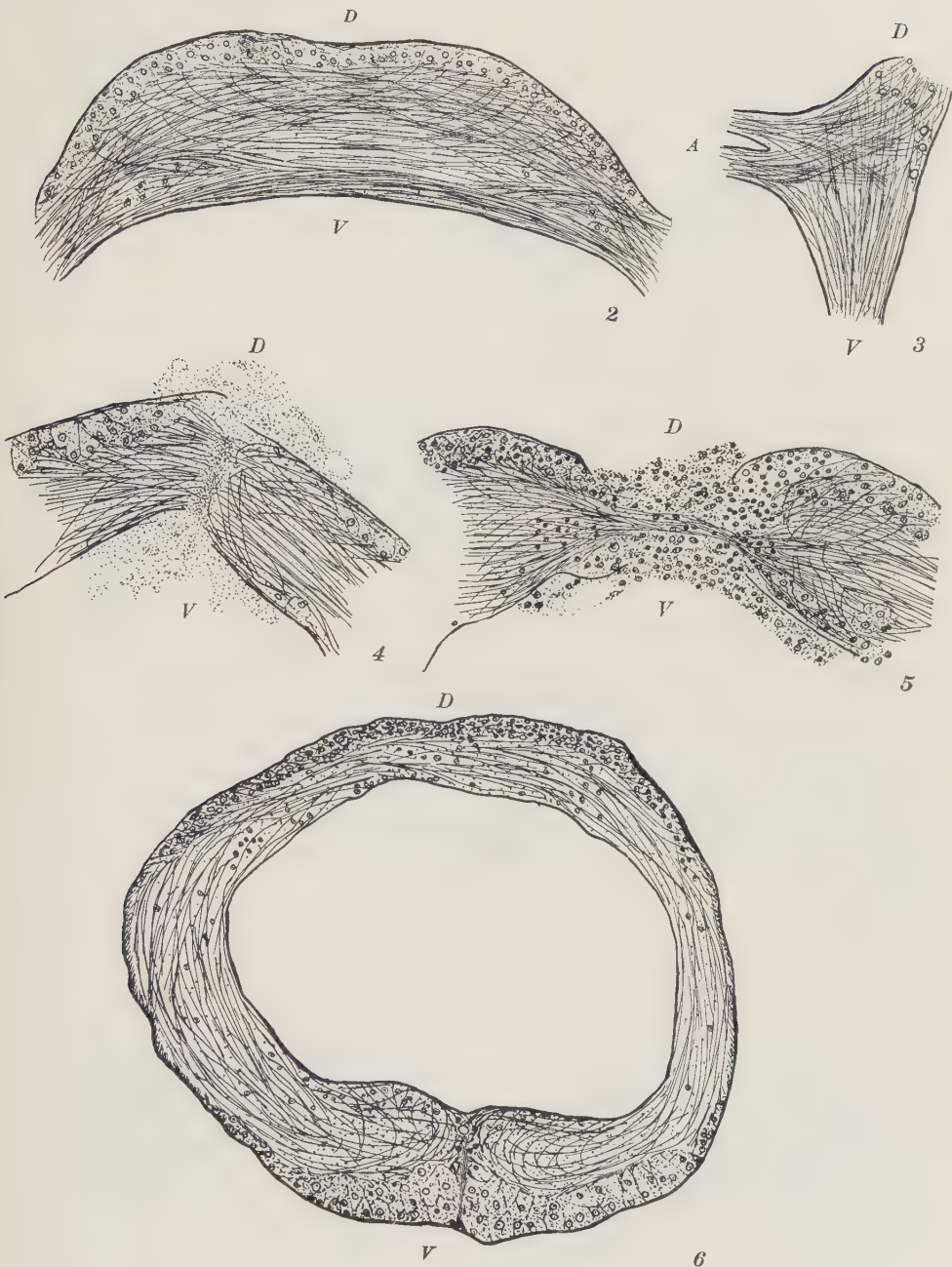


PLATE 2

EXPLANATION OF FIGURES

Figures 7 and 8, regenerating brains; figures 9, 10, 11, and 12, specimens fixed on day following operation.

7 Cross section of brain after one lobe had partly regenerated, showing a small bundle of new fibers extending through the cicatrix from the remaining lobe to the pharyngeal commissure. This specimen had recovered nearly normal negativity. Haematoxylin. $\times 143$.

8 Cross section of brain after transection of one pharyngeal commissure, showing new fibers extending from brain. This specimen was 60 per cent less negative than before the operation. Haematoxylin. $\times 143$.

9 Horizontal section of brain showing presence of few posterior fibers. This brain was pierced through the middle and the fibers are postero-dorsal; the specimen responded normally. Silver impregnation. $\times 143$.

10 Horizontal section of brain cauterized in posterior region showing small number of anterior fibers. This specimen gave normal negative responses. Silver impregnation. $\times 143$.

11 Cross section of brain cut ventrally, showing small number of dorsal fibers. This specimen responded normally. Silver impregnation. $\times 143$.

12 Cross section of brain cut on dorsal side, showing small number of ventral fibers. This specimen responded normally. Silver impregnation. $\times 143$.



VITA

Clifford Ladd Prosser was born at Avon, New York on May 12, 1907. He received his primary and secondary education in the Avon schools and graduated from the Avon High School in 1925. He entered the University of Rochester in 1925 and received the A.B. degree with honors in biology in 1929.

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